CHARACTERIZATION OF PERTUSSIS RISK AND ANTIBODY TRANSFER IN INFANTS IN SARLAHI DISTRICT, NEPAL: A COMMUNITY-BASED PROSEPCTIVE COHORT STUDY

By

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A dissertation submitted to Johns Hopkins University in conformity with the requirements for the degree of Doctor of Philosophy

Baltimore, Maryland

December 2014

Abstract

Background

Pertussis is estimated to cause 2% of childhood deaths globally and is a growing public health problem. Infants are at greatest risk of morbidity and mortality. Maternal vaccination during pregnancy may be effective to prevent pertussis in young infants but population-based estimates of disease burden in infants, an understanding of maternal and infant pertussis antibody levels, and of efficiency of maternal to infant antibody transfer in a low-income South Asian setting are lacking. This dissertation provides a population-based estimate of the incidence of infant pertussis and associated clinical symptoms in Nepal. The timing of infant pertussis vaccination and risk factors for delay in Nepal are examined. The prevalence of maternal and infant pertussis toxin antibody and the efficiency of transfer were estimated.

Methods

The pertussis study was nested within a prospective, community-based, randomized controlled trial of maternal influenza vaccination during pregnancy. In Sarlahi District, Nepal, over a two-year period between April 2011 and April 2013, approximately 3,700 women were enrolled. From birth to 6 months infants were visited in their homes weekly to ascertain if they had experienced any respiratory symptoms or received any vaccinations in the prior week. If any respiratory symptoms had occurred, a nasal swab was collected and tested with a multi-target pertussis PCR assay. A subset of paired blood samples from mothers and infants were collected at delivery and tested for pertussis toxin (PT) antibodies by

ELISA. Infant, maternal, and household characteristics were captured at enrollment, birth and at 6 months follow-up.

The incidence of pertussis from age 0 to 6 months was estimated. Pertussis vaccination coverage and time to vaccination were estimated. Multivariate regression models were used to determine risks associated with vaccination delays. PT antibody levels and the maternal to infant transfer efficiency were estimated. Infant, maternal, and household characteristics associated with non-presence and low levels of PT antibody were calculated through multivariate regression models.

Results

Only 7% of infants had received all three recommended pertussis vaccinations by age 6 months. The incidence of PCR-confirmed *Bordetella pertussis* was 5.2 cases per 1000 infant-years (95% CI, 2.1 – 10.7) and cases were generally mild. The PT infant to mother ratio was 1.1 (95% CI: 1.0 – 1.2). Mother and infant pairs with detectable PT antibody were correlated but the majority of mothers and infants had antibody levels below the level of quantification.

Conclusion

Population-based active home surveillance for respiratory illness identified a low incidence of pertussis among infants in rural Nepal. Nepal's immunization program, which includes 3 childhood whole cell pertussis vaccine doses, appears to be controlling pertussis in infants despite substantial delays in time to vaccination. Maternal and infant PT antibody levels were low. Overall transport was active for mothers with antibody titers above detectable levels and there was

an association between these mothers and their infant PT antibody levels. Maternal immunization could be an important intervention to support infant pertussis immunity before infants are fully vaccinated.

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Acknowledgements

I am incredibly fortunate to have had Dr. Joanne Katz as my adviser both as a master's student and during my doctorate training. The reason the pertussis project has become a reality is due entirely to Joanne's avid support of the initial idea. She helped identify potential sources of funding and worked exceedingly hard with me to submit several grant applications**.** She encouraged my collaboration with research partners with support for my travel to the U.S. laboratory testing sites. Further, she has supported my presentation of this work at scientific research meetings. When there was a unique opportunity to present our work to the Thrasher Research Fund she pushed me to give the presentation to benefit my professional development when she could have easily given it as the Principal Investigator of the study. For almost four years she has made a commitment to meet with me on a weekly basis to review my progress, provide epidemiological and statistical expertise, and to provide mentorship for my development. I have had the unique opportunity as a student to be involved in a project in its entirety, including development of the initial concept, grant writing, implementation, analysis and presentation of results. This truly would not have been possible without her guidance and support.

My gratitude goes to Dr. Subarna Khatry and the entire NNIPS staff for all of the work they have done to support the pertussis study and the full welcome I received during my stay in Nepal. The key staff includes Dr. Subarna Khatry, Shakuntala Singh, Steven LeClerq, Dambar Pandey and the data center team,

Tirth Raj Shakya, Uma Shankar Sah, Shishir var Shrestha, the Auxillary Nurse Midwives, Team Leader Interviewers, and Flu Data Collectors. Supervisor Keshab Prasad Dhakal deserves special thanks for being my primary fieldwork guide in the study area and a patient Nepali language teacher. This work would not have been possible without the women and infants who donated generous amounts of their time to participate in this study. I appreciate Dr. Jim Tielsch and Dr. Luke Mullany's support in including the pertussis research in the NNIPS study area as this project added significant effort to the already bustling NNIPS research agenda.

Several U.S. collaborators who provided laboratory and technical support were critical to this work. Dr. Janet Englund, co-Principal Investigator of the study at the University of Washington, has provided her expertise in pertussis and maternal immunization throughout the study. Her critical review of the research methods and results and sharing of new data has been invaluable. She personally hosted me during my visit to the testing laboratory at the University of Washington and treated me as one of her own trainees during a scientific meeting in Vancouver. Thank you to Dr. Jane Kuypers, Director of the Molecular Virology Laboratory at the University of Washington, who oversaw the pertussis multi-target PCR testing. Two collaborators, Dr. Kathryn Edwards and Dr. Michael Rock at Vanderbilt University, are appreciated for their willingness to conduct ELISA antibody testing, their pertussis expertise, and their patience with the pace of specimen delivery for testing. I thank Dr. Mark Steinhoff of Cincinnati Chil-

dren's Medical Center and Principal Investigator of the maternal influenza trial for his overall support of the pertussis project.

I would like to thank members of my Final Committee of Readers and my Thesis Committee for their input in the final document. I especially want to thank Dr. Saad Omer, who sparked my original interest in pertussis during my master's degree as an adviser and mentor. He involved me in a literature review on the global burden of pertussis and has continued to guide me throughout my vaccine research endeavors.

Funding for this work has come through a constellation of partners who believed in the critical importance of this work to support newborn health. This pertussis research would not have been possible without the generous support of the Thrasher Research Fund through the E.W. "Al" Thrasher Award. Thank you to the Bill and Melinda Gates Foundation for the initial support of the maternal influenza vaccination trial and now their provision of additional funding to continue and expand upon the pertussis research begun through the Thrasher Research Fund Award. Strong appreciation is also due to the donors of several awards, which have supported my academic program including this research. The Clinical Trials Training Program through the National Eye Institute funded me through a pre-doctoral National Research Service Award (NRSA) during the critical period of proposal development.

The following Johns Hopkins Bloomberg School of Public Health school-wide endowed awards provided support for this research: Clements-Mann, R.

Bradley Sack Family Scholarship Award, Procter & Gamble Fellowship, and the David and Elinor Bodian Scholarship Award. The Center for Global Health supported expenses during my field experience through a Global Health Field Research Award. I cannot list these awards without commending Joanne who is a tireless supporter of students. As her advisee she has pushed me to apply for all relevant funding opportunities and has written countless recommendations to support this effort. My successes with these scholarship and grant applications are in large part due to her advocacy for my success.

Lastly, I want to acknowledge my partner George Hughes for his continued support of my academic career. Over 10 years ago he encouraged me to travel to China and Kenya for my first global health adventure despite the lengthy separation this required. Without these formative experiences I would not have had the inspiration to begin my career in public health. Since then he sacrificed his time by moving to Baltimore during my first year of graduate study despite the lengthy commute he endured to teach in Washington, DC. When further opportunities arose away from our home he has been a constant supporter of my temporary relocations understanding the importance of these experiences to my professional growth. Our parallel and complementary trainings in public health and medicine have led to richer understanding of each of our professions. Thank you for your love, patience, and willingness to listen even after a long day's work about the intricacies of my favorite bug, *Bordetella pertussis*.

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CHAPTER ONE

Background and Introduction

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Section 1 - History

Guillaume de Baillou first described a 1578 French pertussis epidemic in a manuscript published in 1640[1]. Baillou wrote, "The symptoms of [*pertussis*] are severe. The lung is so irritated that in its struggle to drive out by utmost effort the cause of irritation, it can neither inspire, nor with any ease expire. The patient seems to swell up, and as if on the verge of suffocation with his breathing obstructed in midthroat." Other descriptions and names of a pertussis-like illness are found dating to Hippocrates in 400 B.C. However, since modern diagnostic techniques were unavailable, we cannot know for certain that these accounts are related to the pertussis we understand today[2]. Recent genetic analysis indicates pertussis likely emerged in humans approximately 500 years ago[3]. In 1679 Thomas Sydenham coined the name "pertussis" to the previously described disease[4]. *Bordetella pertussis*, the causative agent of pertussis, was not isolated until Jules Bordet and Octave Gengou cultured the bacterium in 1906[5].

Previously pertussis was a near universal childhood disease peaking in a cyclical pattern every 2-4 years in developed countries[6]. During the 20th century the United States (U.S.) and Europe experienced a steady decline in pertussis mortality[7,8]. The introduction of the whole-cell pertussis (wP) vaccine in the 1930s accelerated the reduction in childhood pertussis infection and deaths. In the 1990s the U.S. and other developed countries replaced the wP vaccine with the acellular pertussis (aP) vaccine. In recent decades, there has been a pertussis resurgence in high-income countries[9].

Section 2 – Pertussis Burden

Millennium Development Goal (MDG) 4 is to reduce the number of under-5 deaths by two-thirds by 2015 compared to the number of deaths in 1990[10]. Pertussis is estimated to cause 2% of childhood deaths each year in the world[11]. To achieve MDG 4 it will be important to make progress in the prevention and treatment of pertussis in children.

Subsection 2.1 - Children

Pertussis (whooping cough) is a significant cause of childhood morbidity and mortality. The World Health Organization (WHO) estimates that 16 million children contract pertussis each year resulting in 195,000 deaths[11]. The distribution of pertussis, however, is not equal; 95% of pertussis cases are thought to occur in developing countries[12]. A systematic review of the causes of <5 child mortality in 2008 found the regional estimates of proportion of deaths attributable to pertussis as follows: Europe and Western Pacific <1%, Americas 1%, Africa and Eastern Mediterranean 2%, and Southeast Asia 4%[11]. Pertussis suffers from substantial underreporting, which increases with age, making precise burden estimates difficult[13].

The highest quality estimates of childhood pertussis burden are found in aP vaccine clinical trial data from studies conducted during the 1990s testing the aP vaccine in various populations [Table 1.1]. The incidence of pertussis ranged from 0.10 to 11.02 cases per 100 person-years (PY) in children who were unimmunized or who received one or more doses of the wP vaccine[14-20]. One study,

which may provide the most accurate pertussis incidence estimate in children, was conducted in Senegal. In the study children 0-15 years were actively fol-

TABLE 1.1

Pertussis Incidence in Children from Acellular Vaccine Trials

Setting	Vaccination Group	Surveillance	Age	Confirmation Method	# Cases	Person-time at risk	Incidence (cases/100 person-years)
Italy (Greco) 1992-1994	DT	Monthly calls; Evaluate if cough >7 days	>6 weeks	>21 days cough, culture or serological	155	1,768,791 person-days	3.2
	DTP				303	5,325,632 person-days	2.08
	DT			>7 days cough	92	758,646 person-days	4.43
	DTP				211	2,262,810 person-days	3.4
Sweden (Gustafsson) 1992-1995	DT	Call every 6-8 weeks; parents could call if cough illness > 7 days	> 2 months	Culture, serology, and PCR, > 21 days cough	756	103,89 person-years	7.27
	DTP				307	8,215 person-years	3.74
Sweden (Trollfors) 1992-1994	DT	Parents call if anyone cough >7 days	Main follow up >30 days after 3rd vaccination at 1 year	WHO definition	X	X	10.32
	DTP				X	X	2.96
	DT			Cough >= 7 days and meet 1 WHO lab criteria	X	X	11.09
	DTP				X	X	5.13
Sweden (Olin) 1993-1994	DTwP	Rely on physician and parental reporting	>3 months	Cases of pertussis with > 21 days of paroxysmal cough	26	14,977 person-years	0.1
				Cases of pertussis with or without cough	45		0.18
Germany (Stehr) 1991-1993	DTP	bi-monthly calls; parents report if cough > 6 days	>6.5 months	Culture, ELISA, or household contact to confirmed case	X	X	0.6
	DT				X	X	3.4
Senegal (Simondon) 1988-1994	DTwP	Active weekly surveillance by field worker for cough >7 days	0-15 years	-serology, culture, or epidemiology linkage and >21 days cough	162	3,165 person-years	5.12
	DTaP				233	3,193 person-years	7.30
	DTwP			>21 days cough	65	3,165 person-years	2.05
	DTaP				128	3,193 person-years	4.01

lowed (weekly) by a home fieldworker visit to monitor for cough >7 days[15]. The pertussis incidence estimate in this study ranged from 2.05-7.03 cases per 100 PY and varied according the vaccination group (aP vs. wP) and laboratory criteria used for diagnosis (DNA amplification, serology, culture, or epidemiological linkage). In other aP vaccine studies the follow-up was less frequent and some required parental reporting instead of active case finding by field workers, which may have led to underestimates of disease burden[19]. Furthermore, in all of these studies, testing was restricted to those with a cough of minimum 7 days duration. If these children had atypical pertussis, especially the adolescents or youngest infants, this screening threshold may have limited mild pertussis case detection. Lastly, these data are not based on recent cohorts. More recent data suggests that the epidemiology in children has changed with an increased incidence in adolescent populations[21].

Subsection 2.2 - Infants

The majority of studies on the incidence of pertussis in infants <6 months are hospital based or derived from passive surveillance. Reported pertussis cases and deaths based on passive surveillance, seroepidemiology, and hospital studies show the burden of disease is highest in infants less than 6 months[22-30].

A 2001-2004 prospective study in Brazil, Costa Rica, Germany, Singapore, Spain, Taiwan, and Uruguay investigated the incidence of pertussis in infants presenting to pediatric intensive care units[31]. Infants <1 year were eligible for enrollment if they had any of the following symptoms: respiratory failure, ap-

nea, bradycardia or cough accompanied by paroxysms, vomiting, whoop or cyanosis. Twelve percent of infants enrolled in the study had laboratory confirmed pertussis with a mean age of 2.6 (standard deviation (SD) = 2.2) months.

In an early 1990s French pediatric hospital study, where whole cell vaccine was used, the estimated incidence in children was 95 cases per 100,000 PY[22]. In France, a retrospective study conducted in 2000 examined community acquired bacterial causes of death in children 10 days to 18 years[32]. Pertussis was responsible for 13% of the deaths. Furthermore, in infants <2 months of age, pertussis was the leading causative factor of death. Reported cases in King County, Washington State (2002-2007) estimated pertussis incidence at 136 per 100,000 infant population[25].

The reported incidence from the aP vaccine trials [Table 1.1] may provide some indication of the burden in infants <6 months but the applicability of these data may be limited for several reasons. First, most of these studies did not monitor children for pertussis until a certain period after the 1st pertussis vaccination, or more commonly after the final pertussis vaccination. Infants in these surveillance windows are generally older than 4 months and most likely older than 6 months if monitoring commences after a child is fully immunized. Second, these studies did not include unimmunized controls and therefore these incidences are in a semi-immunized and/or fully immunized population, which may underestimate the burden in children too young to be immunized or who are delayed in receiving doses in the pertussis vaccine series. Further, these studies were conducted in the 1990s. Pertussis incidence has substantially increased in recent

years so data from this period may not adequately represent current epidemiology[29,33-35].

In the U.S. infants less than 1 year have the highest rate of reported pertussis at 70.9 cases per 100,000 population[21]. In 2009 the number of pertussis cases in in the U.S. in infants less than 6 months was reported as 126.9 per 100,000 infants[36].

Subsection 2.3 - Adults

Multiple studies have investigated the proportion of prolonged cough illnesses in adults attributable to pertussis with the range approximately 12-32%[4,9,37-47]. Adult serum specimens were tested using enzyme-linked immunosorbent assay (ELISA) and nasopharyngeal specimens were tested by polymerase chain reaction (PCR), direct fluorescent antibody (DFA), or standard culture. Laboratory testing was conducted using at least one or a combination of these methodologies. In one study, 21% of adults with prolonged cough illness presenting to U.S. emergency departments tested pertussis positive (via culture or ELISA)[40]. Another study looking at a U.S. urban population found 12% of adults with prolonged cough >2 weeks tested positive for pertussis using the ELISA method. The investigators estimated the incidence of pertussis in adults in the U.S. is 176 per 100,000 PY (95% Confidence Interval (CI)): 97-255)[38]. An additional population-based study of adults in Minneapolis estimated the incidence in the U.S. is 507 cases per 100,000 PY using a combination of ELISA, culture, and PCR methods (95% CI: 307-706)[37].

One of the best estimates of the incidence of adult pertussis comes from a 1997-1999 U.S. clinical trial of the aP vaccine[48]. Participants, ages 15-65 years, were randomized to receive either the aP vaccine or a hepatitis A vaccine as the control arm. Those enrolled were called every 2 weeks to determine if they had a cough for greater than 5 days. Persons with persistent cough had specimens (nasopharyngeal aspirate and blood) collected for laboratory confirmation. The incidence of pertussis among those in the control arm was estimated to be between 370 and 450 cases per 100,000 PY depending on the pertussis definition utilized.

Use of serology increases the sensitivity for detecting asymptomatic and mild pertussis cases. The adult aP trial ascertained the total number of cases through serologic testing and found that there are 5 asymptomatic or clinically insignificant infected persons for every confirmed case of pertussis[49]. The results showed 1% of the population is infected with pertussis annually. A study of the U.S. National Health and Nutrition Examination Survey (NHANES) in persons ages 10-49 provided a higher prevalence of pertussis infection at 2.98% translating to an annual prevalence of 2,890 per 100,000 persons[50]. A cross-sectional study from China estimated the incidence of pertussis infection as determined from a single serological specimen[51]. The investigators estimated that the incidence of pertussis in persons aged 2-20 years in China was 7,000 per 100,000 PY. Another recent China study found the pertussis incidence to be 9,395 cases per 100,000 PY in those over 7 years[52]. As there is no absolute antibody titer level that correlates with protection or recent infection, these results based on a

single sample overestimate the incidence. Similar to infants, pertussis is resurging in adolescent and adults[21,35,53].

Subsection 2.4 – Asia and Nepal Burden

While community-based data on the incidence of pertussis in developed countries is limited, it is almost non-existent in Nepal and other Asian countries where surveillance is less robust and based on nationally reported cases or seroepidemiology studies[54,55]. Reported numbers of pertussis deaths from passive surveillance are not adequate to measure pertussis morbidity and mortality[55-57]. In the WHO Southeast Asia region the reported pertussis incidence on the 2014 Joint Reporting Form was 2,027 cases per 100,000 PY[58]. An estimated 4% of all childhood deaths in Southeast Asia are due to pertussis according to a global systematic review[11].

A seroepidemiology study in Iran estimated approximately 7% of the population aged 7-35 years had a recent pertussis infection (based on high PT immunoglobulin G (IgG) titers)[56]. Two Chinese studies show a lower prevalence with approximately 7-9 cases per 1,000 population based on serology[51,52]. A pertussis resurgence was found in vaccinated children of Pakistan during recent surveillance (2004-2006)[59] and in Iran[60]. A modeling study from Thailand found no increase in pertussis in recent years[61].

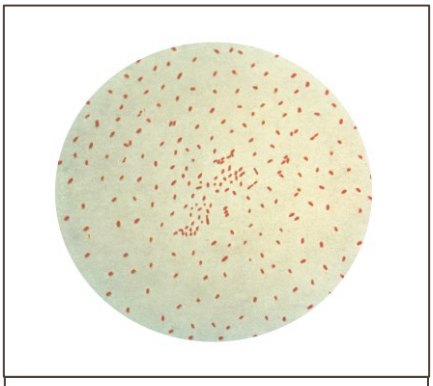
In Nepal specifically, pertussis is estimated to contribute to 2% of childhood deaths based on the aforementioned global systematic review[11]. 3,431 cases were reported in 2013 giving a population incidence of 12,343 cases per

100,000 PY[62]. Reported cases over time have not substantially increased as has been found in other countries.

Section 3 – Microbiology, Pathology, and Immunology

Subsection 3.1 - Microbiology

Bordetella pertussis is a small, non-motile, gram-negative coccobacillus whose habitat is the mucosal layer and cilia of the human respiratory tract [Figure 1.1][12]. *Bordetella* pertussis is a member of the *Bordetella* species, which also includes genetically related *B. parapertussis* and *B. bronchiseptica*[4,63]. *B. parapertussis* causes similar disease as *B. pertussis*, however the symptoms are milder; it is also found in sheep. Non-human animals (dogs, rabbits, horses, pigs) are the typical host of *B. bronchiseptica* although human cases have been documented[2,64]. The difference between these three species lies mainly in differential gene expression and polymorphisms in certain genes[65]. The *Bordetella* species also includes *B. avium* (birds), *B. hinzii, B. holmseii*, *B. trematum*, *B.* petrii, and *B. ansorpii* which are poorly understood but are thought to infect both animals and humans[64-66].

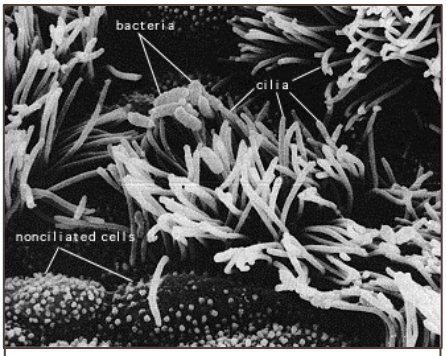


Source: CDC – public health image library #2121

FIGURE 1.1 - GRAM-STAINED PHOTOMICROGRAPH OF *BORDETELLA PERTUSSIS*

Subsection 3.2 Pathology

B. pertussis is transmitted from person to person contact through pertussis-containing respiratory droplets[12]. Once a susceptible host is infected, the bacteria will colonize and multiply in the mucous membranes of the respiratory tract [Figure 1.2][2]. The organism specifically resides on the ciliated epithelial cells of the trachea and bronchi and does not enter the sub-mucosal cells or the bloodstream. *B. pertussis* bacteria secrete toxins, which paralyze the cilia and inflame the respiratory tract. An infected person then has difficulty clearing respiratory secretions[67]. The specific pathway through which *B. pertussis* infects a person, destroys/inflames the respiratory tract, and evades the immune system has not been fully characterized.



Source: http://www.textbookofbacteriology.net/pertussis.html

FIGURE 1.2 - COLONIZATION OF TRACHEAL EPITHELIAL CELLS BY *BORDETELLA PERTUSSIS*

B. pertussis expresses a variety of virulence factors including pertussis toxin (PT), filamentous hemagglutinin (FHA), pertactin (Prn), fimbriae (FIM) type 2 and type 3, adenylate cyclase toxin (ACT), tracheal colonization factor (TCF), tracheal cytotoxin (TCT), dermonecrotic toxin (DNT), lipopolysaccharide (LPS), *B. pertussis* endotoxin, *Bordetella* resistance to killing factor (Brk), heat-labile toxin, and Type III secretion system (bscN)[4,12,68-71]. Depending on its environmental conditions *B. pertussis* may alter expression of certain virulence factors. The majority of these factors are regulated by the *bvg* locus on the *B. pertussis* ge-

nome[2]. While related organisms may secrete many of the same factors, PT is specific to *B. pertussis*[72]. Interestingly, other *Bordetella* species (*bronchioseptica* and *parapertussis*) have the gene for PT, however it is not expressed due to nucleotide polymorphisms in the promoter regions[65].

Bordetella pertussis infection involves attachment, evasion of the host immune system, localized damage, and then systemic effects[73]. Filamentous hemagglutinin, Prn, PT, LPS, TCF, Brk, and FIM are thought to aid in the strong attachment of *B. pertussis* to the epithelial cells of the upper respiratory tract[12]. Pertussis toxin and ACT play a role in helping the organism to evade the host's immune system. Local tissue is damaged through TCT and ACT[73]. Pertussis toxin may cause lymphocytosis or hyperinsulinemia.

Subsection 3.3 - Immunity

There are multiple factors thought to stimulate an immune response to *B. pertussis.* Four of these (PT, FHA, Prn, FIM) are included in various combinations of aP vaccines; not all vaccines contain all four antigens. Pertussis toxin is one of the most important factors in pertussis virulence and is a strong immunogen in the human host[72]. While *B. pertussis* itself does not invade systemically, PT is excreted into the blood causing systemic side effects. In mouse models antibodies to PT protect mice from both intracerebral and aerosol challenges. Pertactin antibodies are crucial for facilitating phagocytosis of *B. pertussis*, which may contribute to their role in immunity[74]. Filamentous hemagglutinin and Prn are

both highly immunogenic and antibodies to these proteins are protective against respiratory but not intracerebral murine challenge model.

During the initial weeks of the infection several immune defense factors enter the lungs including macrophages, dendritic cells, neutrophils, natural killer cells, and T cells[75] Animal studies have demonstrated the importance of cell mediated immunity in clearing pertussis infections.

There is no established serologic correlate of immunity to *B. pertussis*. Studies from the mid 20th century indicated high levels of antibodies to LPS, Prn, and FIM type 2 and 3 conferred protection from pertussis disease. More recent studies show that high antibody levels to Prn and FIM types 2 and 3 are predictive of protection against pertussis disease[74,76-79].

Section 4 – Epidemiology

Prior to the introduction of the wP vaccine, pertussis was widespread [Figure 1.3][7]. Despite high levels of vaccination, pertussis remains an endemic disease throughout the world with periodic epidemic cycles every 3-4 years[7,80,81].

Subsection 4.1 Resurgence

In the pre-vaccine era pertussis was mainly a disease detected in childhood with >93% of reported cases occurring in children <10 years and >80% in children <5 years[6,82]. Since the 1980s there has been an increase in pertussis reporting in the U.S. and other mostly high-income countries, in infants, adolescents, and adults [Figure 1.4][6,21,23,34,83-85]. Outbreaks and epidemic levels of per-

tussis have been reported in Australia[86], Sweden[87], Spain[88], Argentina[89],

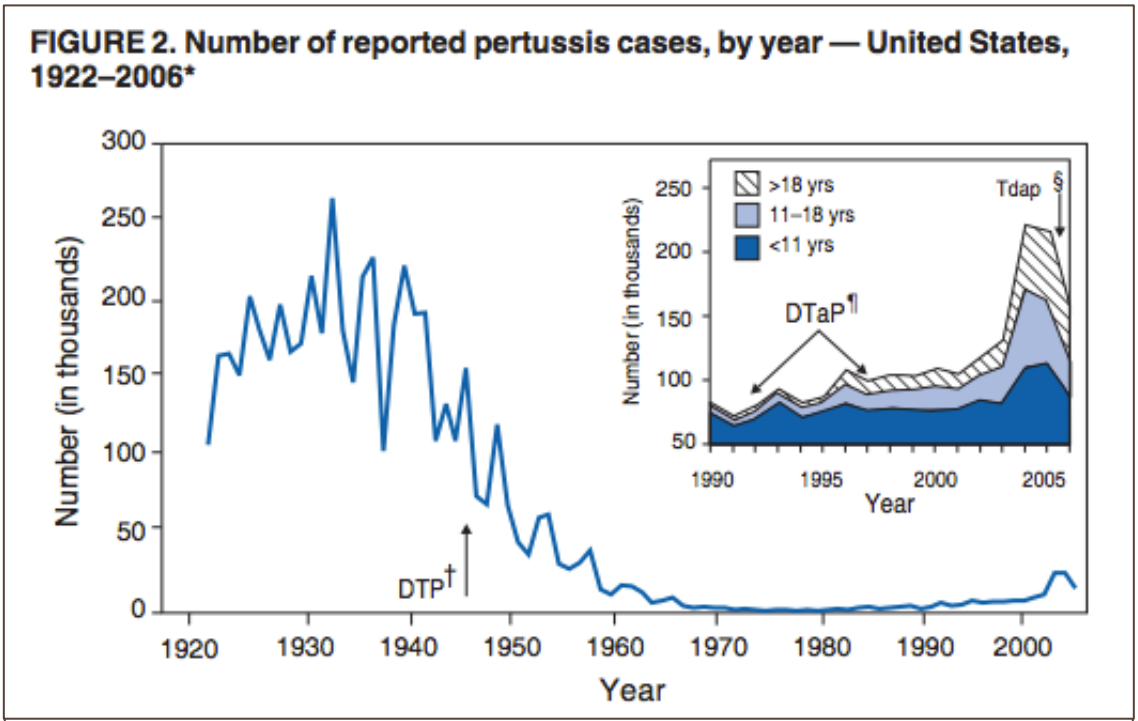


FIGURE 1.3 – U.S. PERTUSSIS CASES 1920-2006

Pakistan[59]. Iran[60], South Korea[30] and the United States[90-92]. In contrast, Thailand has not experienced a pertussis resurgence[61]. A Strategic Advisory Group of Experts (SAGE) on immunization review of evidence from 19 countries however indicated that the resurgence is not global with only 5 of 19 countries showing increased pertussis incidence[93]. Several factors are associated with this increase.

First, substantial evidence exists of worldwide genetic changes in *B. pertussis*, perhaps accelerated by adaptation to pertussis vaccines[3,94-101]. In Australia, strains with polymorphisms in Prn and PT are increasing in prevalence indicating selection for pertussis strains that are not present in current aP vaccines[86].

A retrospective study in the Netherlands examining changes from 1949-2010 found several single nucleotide polymorphisms, which resulted in a switch in the predominant circulating strain[95]. A novel circulating PT promoter allele (ptxP3) was shown to result in higher expression of virulence factors; in a mouse intranasal infection model the ptxP3 variant was significantly better in colonization of the lungs and trachea than ptxP1[98]. Pertactin-deficient strains, first reported in 1994, have dramatically increased since 2010 and are present in several countries[102-105]. Recent data show these Prn-negative strains may be more virulent than strains containing Prn[106]. These Prn-deficient strains arose independently multiple times and not from a single mutation event[107]

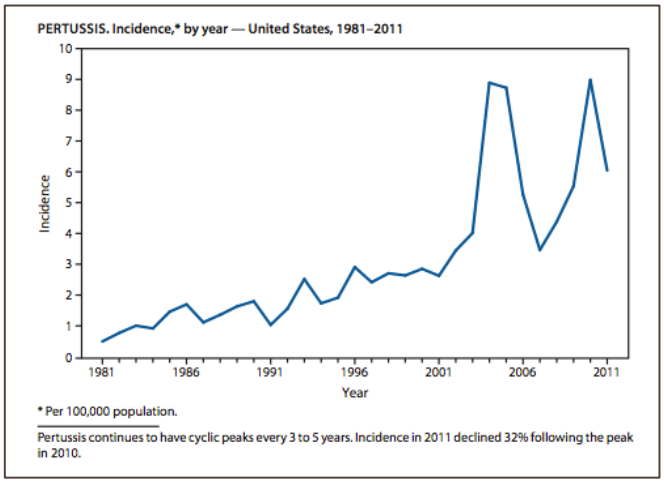


FIGURE 1.4 – U.S. PERTUSSIS INCIDENCE 1981 - 2011

There are differences in vaccine-induced immunity from those who received aP vaccines exclusively versus those who had one or more doses of wP vaccines[92,108]. While the aP vaccine trials in the 1990s showed similar immunogenicity and efficacy for wP versus aP vaccines, follow-up indicates these vaccines are not comparable in long-term protection across age groups[15,81,108-112]. One reason for this may be that wP vaccines contain the entire killed *B. pertussis* organism and therefore a greater number of antigens to stimulate the immune response than the acellular vaccine, which contains only a select few purified an-

tigens[92]. Acellular vaccines and wP vaccines also stimulate the immunity response differently[113,114]. Acellular vaccines induce a Th2 favored immune response and reduced Th1 response for cell-mediated immunity[94]. Whole cell pertussis vaccine generates a broader cytokine response compared to aP vaccines[113]. A study in non-human primates found that while vaccination with aP vaccine prevented severe infection it did not prevent colonization, nor facilitate more rapid infection clearance nor stop transmission to unvaccinated non-human primates[115]. The aP vaccines also elicited a mixed Th1/Th2 immune response compared to the Th1 and Th17 response induced by wP vaccines. Evidence from this study provides some of the strongest mechanistic data to explain the relative vaccine effectiveness inferiority of aP versus wP. Similar data in mice showed aP vaccines were not able to limit shedding or disease transmission compared to wP vaccines[116]. A current SAGE review found 4 of the 5 countries experiencing resurgence were using aP vaccines exclusively[93]. In the one country using wP vaccine other factors were thought to be the primary contributors to the pertussis resurgence such as increased surveillance, changes in laboratory methods, and low vaccination uptake.

Waning immunity from both wP and aP vaccines might also contribute to the increase[53,115,117].

Next, especially in high-income countries such as the United States, there has been increased resistance to vaccination leading either to non-vaccination or delays in vaccination[25,118]. Data from California indicate that non-medical exemptions for pertussis vaccination are associated with increased pertussis risk[119].

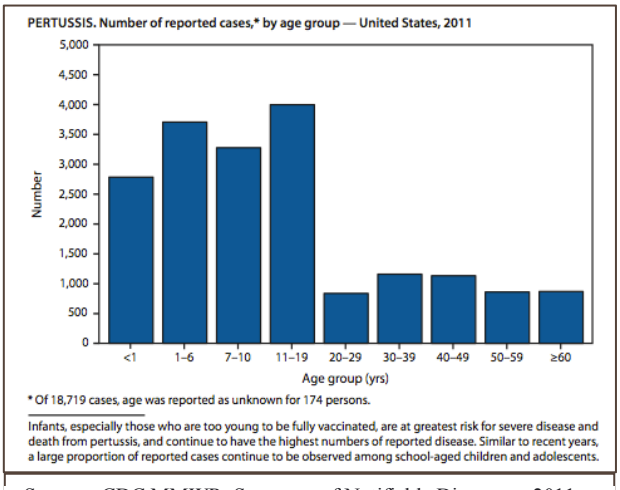
Advances in and greater availability of pertussis laboratory diagnostics including serology and PCR may have led to increased pertussis detection[34,93].

Finally, there may be greater awareness today of pertussis, especially in older persons, increasing the chances that pertussis may be suspected as a differential diagnosis in those presenting with prolonged cough[34].

Subsection 4.2 – Risk Factors

4.2.1 Age

Infants have the highest incidence of pertussis and are at the greatest risk of morbidity and mortality from pertussis compared to any other age group [Figure 1.5][23,24,120]. In the U.S., during the period 2000-2006, infants <1 year contributed to 93% of pertussis-related deaths[83]. Among infants <1 year, infants <6 months experienced the highest burden of disease with the mean age of infection from one hospital study at 2.6 months and another study showing highest burden in those <3 months [Figure 1.6][7,26,31,121,122].



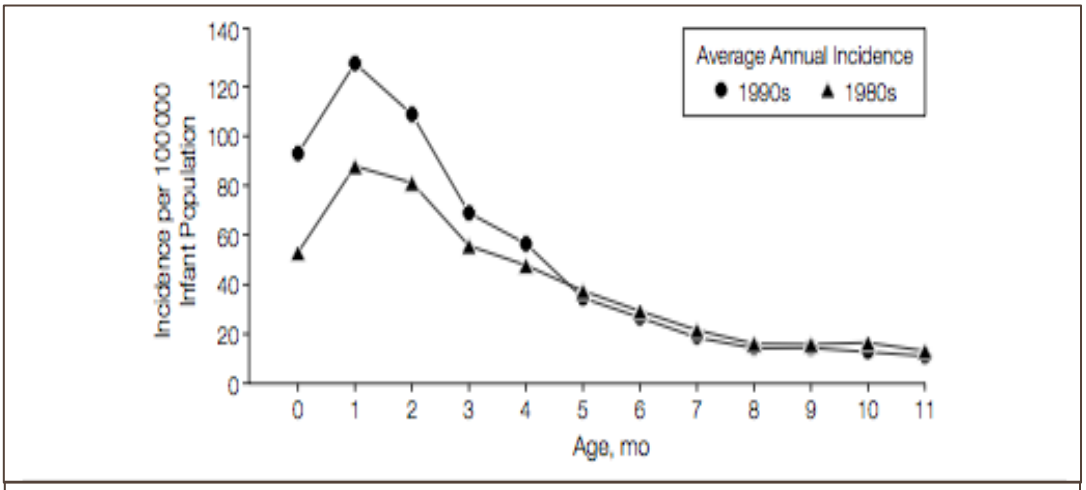
Source - CDC MMWR, Summary of Notifiable Diseases – 2011

FIGURE 1.5 – U.S. NUMBER OF REPORTED PERTUSSIS CASES BY AGE GROUP 2011

Since the 1980s the incidence of pertussis in infants in the U.S. has increased[24].

In addition to infants, serologic studies found peaks in pertussis-associated antibodies in in a period immediately following the final childhood vaccine administration and again in adolescence[50,51,123-125]. In Nashville, adolescents ages 13-17 years had the highest pertussis antibody titers compared to other age groups[125]. In the U.S. APERT study 15-20 year old participants had the highest PT IgG antibody concentrations[124]. A study in China also found 2 age-related peaks in the distribution of antibody titers; one peak at 6-8 years, which is probably a result of recent receipt of the final pertussis vaccine dose and an additional peak in the 12-20 year range most likely indicative of recent natural infection due to waning immunity[51]. South Korean national surveillance found a shift in the age distribution to adolescents[30].

For adults, a study of U.S. healthcare workers found annual serological evidence of infection in 1.3% of medical residents and 3.6% of emergency department staff[126]. A study of University of California Los Angeles female



Source: Tanaka JAMA, Trends in Pertussis Among Infants in the United States, 1980-1999

FIGURE 1.6 – U.S. NUMBER OF REPORTED PERTUSSIS CASES <1 YEAR 2011

healthcare workers found a much higher annual rate of infection ranging from 24% - 43%, however the researchers did not measure antibodies to PT, the most specific antibody for pertussis infection[127]. Additionally they included increases as low as 1.9 fold to represent a recent infection, which may have lower specificity than a more stringent threshold.

4.2.2 Sex

Age modifies the relationship between sex and pertussis with almost equal incidence between sexes in children <1 year. As age increases, the female to male case ratio increases[8]. For those <1 year South Korean and Tunisian surveillance showed an increased incidence in males compared to females[26,30]. In Japan similar incidence was found between the sexes[27].

Overall, females have a slightly elevated risk of pertussis compared to males[8,9,82,128]. In the U.S., females have a slightly higher infection rate compared to males (6.6 vs. 5.4 cases per 100,000 PY) [21]. In Wales and England from 1945 to 1982 the incidence of pertussis was higher in females than in males with 0.88 males cases for each female case (95% CI: 0.84-0.92)[7]. The reason for these sex differences in incidence is unknown.

4.2.3 Ethnicity/Race

In King County Washington, infants who were in ethnic and racial minority groups had higher risk for pertussis compared to while infants[25]. Black infants

had 3.4 (95% CI: 2.6 – 4.4) times the risk of pertussis compared to white infants. Hispanic infants are also at higher risk than non-Hispanic infants[24,25].

4.2.4 Maternal and household characteristics (for infants)

A 1993 retrospective, matched, case-control study from Chicago found no difference between cases and controls with regards to sex, birthweight, age, mother's education, or number of persons in the household or per room[129]. Controls were more likely to receive public assistance compared to cases. In another study young maternal age was a risk factor for infant pertussis with an infant's risk of pertussis increased by 11% for every 1-year decrease in maternal age[129]. Maternal history of antecedent cough was also associated with infant pertussis. In U.S. Centers for Disease Control and Prevention (CDC) reported pertussis deaths, a substantial proportion (51%) were in infants born before 37 weeks, and 29% were in infants born before 35 weeks[23].

4.2.5 Co-Infections

Co-infection with pertussis is common. In a 1990s study of co-infections during a pertussis clinical trial, 21% of children infected with pertussis were also infected with other organisms including *Mycoplasma pneumonia* and *Chlamydia pneumonia*[130]. One study in hospitalized infants in England found that among children who had pertussis, 33% were co-infected with respiratory syncytial virus (RSV)[131]. In Finland, 67% of infants <6 months hospitalized with pertussis were also infected with RSV[132]. A smaller proportion of infants were co-infected with

rhinovirus and influenza A. Another study in Finland found that 8% of infants hospitalized with RSV also had pertussis infection[133]. A multi-country study of infants found that 21% of infants with pertussis were co-infected with another organism (9% RSV, 9% influenza B, 3% influenza)[31]. In the U.S., among reported pertussis infant deaths, 18% were co-infected with RSV, and 15% were infected with other viruses including influenza, parainfluenza, *cytomegalovirus*, and adenovirus[23]. Bacterial pathogens were also found in cultures from blood or autopsy lung tissue in 15% of infants such as *Streptococcus pneumonia* and *Haemophilus influenzae*.

Co-infection with *B. parapertussis* was found in approximately 10% of Tunisian infants infected with *B. pertussis*[26]. An outbreak study in Ohio also found a small percent of co-infections with *B. holmseii*[90].

4.2.6 Pre-existing conditions

One study found patients with asthma had a 17% increased risk for pertussis[134].

4.2.7 Season

It remains unclear whether pertussis has a distinct seasonality with some experts claiming no seasonality while others argue for differing periods of peak pertussis transmission[7,8,80]. Peaks in winter or spring were seen in England and Wales in the pre-vaccination period. In Japan, U.S., and Hungary summer peaks of pertussis have been documented[82,135]. Two small studies in the early 1970s in

India found seasonality of pertussis infection with increased infections from November to June, which is consistent with a more recent 2009 study[136]. A 1959-1966 study in the U.S. found a peak in July and August and a nadir December through March[128]. In the 1990s infants had a peak incidence in July and August and adolescents in the fall[23,24]. Immunization uptake rates and school opening dates may confound seasonality findings[80]. A South Korean study found increased pertussis cases between May and November[30].

Subsection 4.3 Transmission

Pertussis is transmitted through airborne respiratory secretions from person to person. The bacterium is fastidious and survives externally for just a few hours[4]. Humans are the only known reservoirs of pertussis disease[8,137]. Pertussis is highly infectious with a >80% secondary attack rate in susceptible persons[138]. Recent estimates of the basic reproductive number are in the range of 5 to 11[13,139]. In a non-human primate model those vaccinated with aP vaccines were still able to transmit pertussis to other close contacts[115]. Household contacts are a main source of exposure, especially for young infants[22,23,25,129,140,141]. Parents are the most commonly identified source of infection for infants followed by siblings and other household members[28,142-146]. In a multi-national study looking at sources of infant infection, mothers constituted 50% of the transmitters followed by siblings (17%)[31]. A United Kingdom study of infants hospitalized with pertussis found 42% had a parental source of infection and 27% were infected by an older sibling[131]. An Australian study found siblings constituted 36% of pertus-

sis infection sources followed by mothers (15%), other family members (21%) and friends[147]. A literature review summarized that mothers, fathers grandparents, and siblings, were the source of pertussis infection 39%, 16%, 5%, and 16-43% of the time, respectively[148]

Subsection 4.4 Carrier State

Prior to PCR testing, culture was the primary method for pertussis diagnosis. Using culture detection methods, pertussis is exceedingly rare or non-existent in healthy individuals but the sensitivity of culture is quite low[4,137,149-151]. In Sweden, 391 healthy close-contacts of pertussis cases were tested by culture for pertussis[152]. Only one healthy contact tested positive for pertussis indicating healthy carriers may play little to no role in pertussis transmission.

However, diagnostic advances, namely PCR, allowed for detection of smaller numbers of pertussis organisms than possible through culture. Results using PCR have demonstrated carriage in otherwise healthy contacts[143,149,153,154]. For example, in an outbreak among Israeli soldiers, investigators found 20% of healthy contacts were PCR positive for pertussis during the outbreak[149]. However, at least one study using PCR found no asymptomatic carriers in pre-school children exposed to pertussis as all children who tested positive by PCR exhibited at least a mild cough[155].

Non-human primates who had previously experienced a pertussis infection had no colonization when challenged with *B. pertussis*[115]. On the contrary, wP and aP vaccinated non-human primates were subject to colonization after *B.*

pertussis challenge peaking around 2 weeks and were able to transmit the bacteria to naïve cage-mates. Non-human primates vaccinated with wP vaccine cleared the colonization quicker than those vaccinated with aP vaccine (18 versus 30 days).

Section 5 – Clinical Presentation

Subsection 5.1 - Children

Before the introduction of the pertussis vaccine young children were the primary age group infected with pertussis and they exhibited the classical pertussis clinical presentation. Pertussis begins with a 7-10 day incubation period (range 5-21 days)[156,157]. It then progresses through three phases: catarrhal, paroxysmal, and convalescent[66]. During the catarrhal phase, lasting 1 to 2 weeks, an infected person experiences symptoms similar to those of the common cold such as rhinorrhea, sneezing, and intermittent cough; fever is generally not present. However, instead of resolving, the disease progresses to the paroxysmal stage, characterized by spasmodic coughing followed by an inspiratory whoop and sometimes vomiting. The coughing episodes may be grouped together with periods of non-coughing followed by heavy paroxysmal coughs[73]. This period usually lasts 1 to 6 weeks but may extend for 10 weeks. Eventually symptoms begin to wane as a person enters the convalescent phase. Complications of the disease may include hospitalization, bronchopneumonia, seizures, acute encephalopathy, pneumonia, and death.

Children who have a history of pertussis vaccination are significantly less likely to have severe morbidity and recover more quickly than their unvaccinated counterparts[158-160].

Subsection 5.2 – Infants

Infants usually experience symptoms atypical from the "classic" pertussis presentation with a shorter catarrhal stage, increased apnea, and sometimes the absence of the classic whoop[157,161]. Infants with pertussis are found to exhibit some of the following symptoms: paroxysmal cough, post-tussive vomiting, apnea, cyanosis, inspiratory whoop, wheezing, bradycardia, and poor feeding[25,162,163]. The atypical presentation may lead to delays in pertussis laboratory testing and pertussis-specific treatment.

Pertussis is most serious in children younger than 6 months, especially in preterm infants and under-immunized infants. Infants who have had fewer pertussis vaccinations are more likely to be hospitalized for pertussis compared to infants of similar age[24]. Complications in infants include pneumonia, apnea, profound lymphocytosis, pulmonary hypertension, bronchiolitis, seizures, encephalopathy, conjunctiva bleeding, and death[23,83,164]. Infants younger than 2 months have a 1% case fatality rate that drops to 0.5% in infants less than 1 year. Older infants are more likely to experience whooping and vomiting and less likely to experience apnea or be hospitalized[24]. Persons who contracted pertussis as infants have a greater risk of having asthma and respiratory infections compared to similar infants with no pertussis history[165].

Subsection 5.3 - Adults

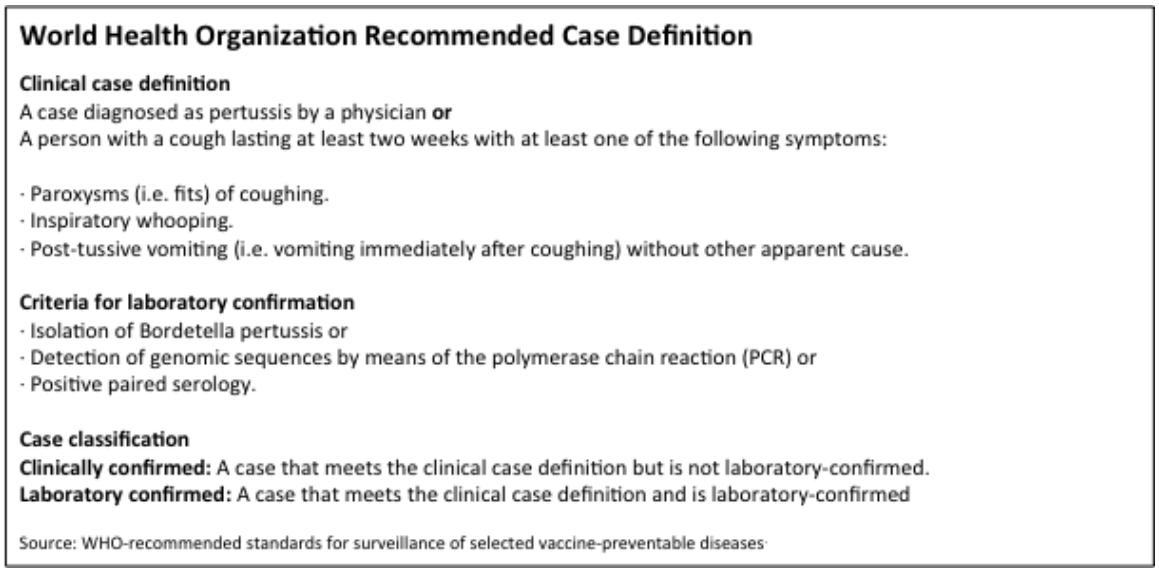
Adults have a clinical presentation of pertussis that is milder than that seen in children and is usually non-specific[40]. Detection of pertussis is limited in adults as they usually come for treatment weeks into the illness when the organism is more difficult to isolate. The catarrhal phase is usually mild or absent in adults due to previous natural of vaccine-induced exposure to pertussis[166]. Adults may experience post-tussive emesis, syncope, problems sleeping, incontinence, fractures of the rib and pneumonia[157].

Section 6 – Diagnosis

Subsection 6.1 - Clinical

Clinical diagnosis of pertussis is difficult as pertussis encompasses a broad range of symptoms and some pertussis may manifest atypically. In 1991 the WHO created an expert panel to develop a consensus case definition of pertussis in anticipation of the upcoming efficacy trials for aP vaccines[72]. The WHO definition required a paroxysmal cough of at least 21 days plus confirmation through either laboratory methods or an epidemiologic link. While this definition helped to standardize the subsequent trials it still led to difficulties in interpretation of results. Use of WHO's definition led to differential sensitivity of case detection in unvaccinated versus vaccinated populations. *B. pertussis* was more likely to be detected in unvaccinated populations and therefore it artificially raised the demonstrated efficacy of the vaccines. The current WHO definition includes clin-

ically and laboratory confirmed cases [Figure 1.7][167]. Clinically confirmed cases must be either diagnosed by a physician or have a cough of at least 14 days (shorter period than pervious definition) plus an additional pertussis-related symptom. A laboratory confirmed case must have laboratory confirmation by culture, PCR, or positive paired serology in addition to meeting the clinical case definition.



World Health Organization Recommended Case Definition

Clinical case definition
A case diagnosed as pertussis by a physician **or**
A person with a cough lasting at least two weeks with at least one of the following symptoms:

- Paroxysms (i.e. fits) of coughing.
- Inspiratory whooping.
- Post-tussive vomiting (i.e. vomiting immediately after coughing) without other apparent cause.

Criteria for laboratory confirmation
- Isolation of Bordetella pertussis or
- Detection of genomic sequences by means of the polymerase chain reaction (PCR) or
- Positive paired serology.

Case classification
Clinically confirmed: A case that meets the clinical case definition but is not laboratory-confirmed.
Laboratory confirmed: A case that meets the clinical case definition and is laboratory-confirmed

Source: WHO-recommended standards for surveillance of selected vaccine-preventable diseases

FIGURE 1.7 – WHO PERTUSSIS CASE DEFINITION

Subsection 6.2 - Laboratory

Determining who has pertussis is difficult. In many areas laboratory and technical capacity to test for pertussis is limited or non-existent. Non-specific laboratory findings may include leukocytosis due to lymphocytosis or thrombocytosis[23,128]. A chest radiograph may be normal or indicate some irregularities, which include peribronchial cuffing, perihilar infiltrates, interstitial edema or atelectasis[23,168,169]. Specific laboratory findings may also be difficult to interpret, as they

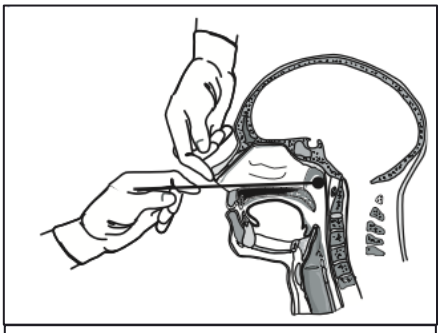
are prone to low sensitivity depending on specimen collection method, collection timing, and testing method. Furthermore, most of the assays lack standardization for comparability between testing centers[170,171].

6.2.1 Direct Isolation

6.2.1.1 Specimen Collection

The CDC and WHO recommend collection of pertussis specimens through either nasopharyngeal swab (NPS) or nasopharyngeal aspirate (NPA) although the aspirate provides higher yields[66,172-176]. Studies to date consistently cite that throat and nasal swabs (NS) have unacceptable rates of recovery. However, this guideline was established when culture was the gold standard in testing methodology. No published studies to date have used NS alone or specifically in comparison to NPA or NPS for pertussis testing[131]. One Australian study included NPA, throat swabs, and NS specimens for PCR pertussis detection[177]. While 85% (445) of specimens were NPA, some were throat swabs (71) and nasal swabs (5). Surprisingly, NS had the highest percent positive rate (40%) compared to 30.1% for throat swabs and 9.2% for NPA. While the numbers were too low to draw definite conclusions regarding comparison of collection methods, it demonstrated that pertussis may be detected from NS. An unpublished (submitted) manuscript found similar sensitivity in detecting *B. pertussis* comparing posterior nasopharyngeal swab collection with mid-nasal swab collection[178].

Some studies have looked at differences in sensitivity and specificity for NPS versus NS for other respiratory pathogens[179]. Meerhoff found NS to have reduced sensitivity (67%) compared to NPS (92%) using a consensus gold standard (positive in either test). For RSV and rhinovirus the reduced sensitivity was significant, 51% and 75%, respectively. In Guinea-Bissau researchers found 27-32% reduced sensitivity of NP compared to NPS for detection of RSV[180]. A study in Finland found NPS to have comparable sensitivity to NS specimens with the exception of RSV[181]. Another study comparing combination nose-throat swabs to NPS also found comparable sensitivity between methods for respiratory viruses[180]. However, the ideal specimen collection location is organism dependent and these differences may not be comparable to differences seen for pertussis.



Source: CDC Vaccine Preventable Disease Surveillance Manual, 5th Edition, 2011

FIGURE 1.8 - PROPER TECHNIQUE FOR OBTAINING A NASOPHARYNGEAL SPECIMEN FOR ISOLATION OF *B. PERTUSSIS*

A diagram of the proper collection method is shown in Figure 1.8. Nasopharyngeal swab specimens are collected by (1) inserting a swab through the nostril into the nasopharyngeal cavity, (2) holding the swab for 10 seconds and turning, and then (3) removing the swab[156]. Nasopharyngeal aspirates are collected in a similar manner but instead of a swab, a small tube connected to a mucus trap is inserted while secretions are aspirated.

6.2.1.2 Culture

The gold standard for *B. pertussis* testing is the culture of nasal secretions due to culture's superior specificity compared to other testing methods[66,156,172]. However, the sensitivity of culture is quite low (15-60%). After the first few weeks the sensitivity continues to decrease for the duration of the disease[182]. Moreover, treatment with antimicrobials, history of previous pertussis vaccination, or non-NPA/NPS specimens will also decrease the sensitivity. Culture on infant specimens yields the highest sensitivity compared to older age groups[66]. Culture remains useful in typing of *B. pertussis* and testing for antibiotic resistance although this is uncommon[68].

Once the specimen is obtained, plates containing media such as RL medium or Bordet-Gengou are inoculated with the pertussis specimen[66,172]. The recovery rate decreases commensurately with the length between specimen collection and plating. Colonies will start to appear by day 3 but culture incubation should continue for at least 7 days. *B. parapertussis* grows more rapidly than *B. pertussis* but both will generate small colonies (approximately 1mm in diameter)[66].

6.2.1.3 RT - Polymerase Chain Reaction (PCR)

Real-Time polymerase chain reaction (RT - PCR) assays have substantially improved pertussis diagnostic capabilities[183]. Polymerase chain reaction has superior sensitivity compared to culture due to its ability to amplify small segments of DNA and reduced susceptibility to interference from antimicrobial

therapy[184]. Moreover, results can be obtained relatively quickly in 1 to 2 days. PCR is most sensitive when used on specimens collected within the first three weeks of symptoms and sensitivity generally declines thereafter[173,185]. Polymerase chain reaction is more sensitive for samples obtained from children compared to adult specimens[156]. Compared to culture PCR also has higher sensitivity as the disease progresses and is more sensitive for mild pertussis cases.

Polymerase chain reaction for *B. pertussis* was developed in 1989, however standardization of assays across laboratories has been limited and no Food and Drug Administration (FDA) approved standardized PCR kit exists[68,183,186]. Polymerase chain reaction can be prone to contamination and difficulties with transport, so careful technique must be used from specimen collection through detection. Initial pertussis PCR assays amplified only a single gene sequence, such as insertion sequence IS*481*, with potential for false-positives and false-negatives[184,187,188]. These PCR assays yielded sensitivity and specificity as high as 95% and 99.3%, respectively[174].

Recent developments have improved the sensitivity and specificity of testing through multi-target real-time PCR[186,189]. In addition to IS*481*, other targets include IS1001, IS1002, h-IS1001, ptx, and recA[66]. Compared to single-target PCR, triple-target PCR (IS*481*,ptx and recA) increases the proportion of positive findings by 1.25 fold, and double-target increases positive results by 1.10 – 1.24 fold[186].

6.2.2 Indirect Detection

6.2.2.1 Enzyme-linked immunosorbent assay (ELISA)

Enzyme-linked immunosorbent assay (ELISA) may be used to diagnose pertussis either through a single serum sample or through positive paired serology[68]. After infection, antibodies to *B. pertussis* may be detected within 1 to 2 weeks. *B. pertussis* primarily elicits IgG antibodies although IgA antibodies are also generated. After infection, antibody levels fall below a defined threshold by 4 to 5 months on average and for the vast majority of patients by 1 year[190]. Antibody testing cannot differentiate between recent infection and recent vaccination. Serologic assays most commonly include testing for PT and FHA, which also may be supplemented with Prn and FIM[76,191,192]. Pertussis toxin is the only antibody specific for *B. pertussis* as other antibodies may cross-react with similar species (e.g. *B. parapertussis* and *Haemophilus influenzae* elicit FHA response)[156]. Pertussis toxin is therefore the antigen of choice for antibody detection[50]. There is no standard ELISA pertussis assay, making comparability between laboratories is difficult[193-195]. Recent efforts have worked to standardize ELISA PT quantification between laboratories[196,197]. One group has recently developed a rapid ELISA to detect antibodies to PT, FHA, Prn, Fim2, and Fim3[198].

No correlate of protection or immunity exists and therefore pertussis is most accurately diagnosed with positive-paired serology from acute and convalescent samples[68]. The acute specimen should be collected as early as possible, ideally within the first two weeks, and the convalescent sample 4-6 weeks there-

after. A 3-4 fold increase in antibody levels is generally accepted to indicate recent infection. Positive paired serology is more specific than a single sample diagnostic test[156]. Serology is considered the most sensitive of all pertussis diagnostic techniques[50].

Many, especially adolescents and adults, present late in the illness when antibody levels have already risen and so paired specimens are not available. Paired serology diagnosis is also less useful; a definitive result is usually available near the conclusion of the disease and therefore is of little utility in guiding treatment of the patient[68]. A single antibody specimen taken at least 2 weeks into the illness may be used but has lower sensitivity and specificity than paired serology and is not useful if a person has been vaccinated in the preceding two years. A PT IgG antibody test above 100 – 125 ELISA units/mL (EU/mL) may indicate recent infection. A study in the Netherlands found a value of 100 EU/mL to have 89.9% sensitivity and 99% specificity when compared to a gold standard of a greater than 4-fold rise in antibody titers[199]. Baughman and colleagues used a mixture model to develop a PT IgG cut-off point of 94 EU/mL[50]. Applied to a previous study in Minnesota the diagnostic sensitivity and specificity of this level was 80% and 93%, respectively using culture as the gold standard comparison.

6.2.2.2 Direct fluorescent antibody technique (DFA)

Direct fluorescent antibody technique (DFA) is another method used to detect *B. pertussis* although it is not commonly used. Advantages of DFA are its

ability to obtain results quickly and its high specificity (99.6%)[172]. Disadvantages are that it requires specialized training and has low sensitivity.

Section 7 - Treatment

For *B. pertussis* infected infants and children, supportive care to minimize adverse effects is most important. A systematic review concluded that antimicrobial treatment has no effect on the course of the disease, however in many of the studies treatment was not started until later stages of the disease[200]. Some studies show antimicrobial therapy is an adequate treatment for patients with pertussis, however its utility is limited to early in the disease course and will have no effect if given at more advanced, paroxysmal stages of the disease[201,202]. As laboratory confirmation is not immediate, it is recommended that a case with high clinical suspicion of pertussis be presumptively treated with antimicrobial therapy. Treatment has also been shown to eliminate carriage of pertussis in the nasopharynx and therefore it is useful in reducing transmission of disease to susceptible persons even if it cannot change the clinical course of disease in those already infected[203]. If antibiotics are indicated, macrolide antibiotics (e.g. azithromycin) should be given. A course of treatment may vary from 5-14 days depending on the specific antibiotic. Prophylactic treatment may also be given to close contacts to minimize the spread of disease although limited evidence indicates this is not effective[204].

Section 8 - Prevention

Subsection 8.1 Natural Infection

Previously, pertussis infection was thought to confer near lifelong immunity[8]. However recent studies indicate that neither natural infection nor vaccination provides complete or long-lasting protection. Modeling of pertussis disease in 1940 found that on average an adult could expect to experience 2.3 cases (mostly mild) of pertussis in his or her lifetime[205]. More recent studies in the Netherlands and Senegal have documented re-infection in children. In the Netherlands, 4 children had a serologically confirmed 2nd pertussis infection with the second infection occurring between 3.5 and 12 years after the first infection[206]. In Senegal recurrent infections were documented in approximately 2.5% of children with a mean duration between infections at 7.1 years in non-vaccinated and 5.1 years in vaccinated populations[207]. Experts now believe that infection-acquired pertussis immunity persists 7-10 years[208].

Confirming duration of immunity is difficult as there is no established serological marker of immunity and antibodies to *B. pertussis* may drop below detectable levels after 2 years despite persisting immunity[208]. In addition, exposure to *B. pertussis* without clinical disease may boost immune response leading to an overestimate of the protective immunity conferred by pertussis disease.

Subsection 8.2 Vaccines

The WHO estimates that since the end of the 1980s, 80% of children worldwide have received pertussis vaccine and pertussis vaccination prevents about 38 million cases and 600,000 deaths annually[209]. Pertussis vaccines are available in two formulations: whole cell (wP) [DPT] and acellular (aP) [DTaP and Tdap]. DPT and DTaP are given to children and Tdap is given to adolescents and adults.

8.2.1 Whole-cell pertussis vaccines

8.2.1.1 Development

Soon after the 1906 discovery of *B. pertussis* as the causative agent of pertussis, researchers attempted to develop a vaccine against the disease[210]. In 1933 Madsen reported efficacy of his vaccine formulation against pertussis morbidity and mortality in two pertussis epidemics[211]. In the 1930s and 1940s large randomized controlled trials were conducted to examine the efficacy of the vaccines. Efficacies between these early vaccines varied tremendously ranging from 53% to 91%[210]. Pertussis vaccines came into routine use in the U.S. and elsewhere in the 1940s and 1950s. Whole cell pertussis vaccines are composed of formalin-inactivated pertussis cells[67].

8.2.1.2 Efficacy

Studies to measure the efficacy of the vaccines continue to yield varying results[195]. Explanations for the differences include variable vaccine formulations

and ingredients, study design (case-control, cohort, secondary-attack rate, randomized-control trial), case ascertainment methods and definition, strain of circulating pertussis, dose and timing of vaccinations, and age of participants among other factors. A systematic review found the pooled efficacy of pertussis vaccines was 78% but efficacy remained heterogeneous between vaccine formulations[212].

8.2.1.3 Duration of Immunity

No study has specifically looked at the duration of immunity following wP vaccination but indirect estimates are that immunity persists for 4-12 years[182,208,213,214]. Protection against pertussis disease persists longer than protection against pertussis infection as asymptomatic infections are reported to have occurred within 1 year following wP vaccination[215].

8.2.1.4 Safety

Several local and systemic adverse reactions are associated with wP vaccines[216]. Local adverse events include erythema, induration, swelling, and pain at the vaccination site. Associated systemic effects include fever, drowsiness, prolonged crying in children and anorexia. Rare adverse events include convulsions, hypotonic hyporesponsive episodes, and acute encephalopathy[12]. Local reactions increase with age and as a result wP vaccines in the form of diphtheria, pertussis, and tetanus (DPT) are only given to children. Due to safety concerns with the wP vaccines, aP vaccines were developed in the 1980s, which have less reactogenicity and an improved safety profile.

8.2.2 Acellular pertussis vaccines

8.2.2.1 Development

In 1981 the first aP vaccine was developed in Japan[12]. The superior safety profile of the aP vaccines led to their adoption in most of the developed world. Acellular pertussis vaccines contain a mixture of purified pertussis antigens. They differ in the composition and quantity of antigens[12]. Components included in aP vaccines include PT, FHA, Prn, and FIM type 2 and 3. Filamentous hemagglutinin, Prn, and FIM type 2 and 3 are all surface proteins, which promote adhesion to the tracheal epithelial cells[65]. Pertussis toxin is an A/B (2-sub-unit exotoxin) that enters the host cells and causes a cascade leading to increased cyclic adenosine monophosphate (cAMP) production.

Adding to antigenic differences, each manufacturer purifies antigens from distinct bacterial clones and the detoxification, purification, preservatives and adjuvants used are variable[12]. Acellular pertussis vaccines are usually combined with diphtheria and tetanus toxoids although sometimes, additional antigens are included in combination vaccines, such as hepatitis B (HepB) or *Haemophilus influenzae* type b (Hib). There is no pertussis only vaccine formulation. Pediatric (DTaP) and adult formulations (Tdap) are available with the adult versions containing lower amounts of diphtheria and tetanus toxoids. In the U.S., the pediatric version was licensed in 1991 and the adult version was licensed in 2005[67].

8.2.2.2 Efficacy

In the late 1980s and 1990s several studies were conducted to measure the efficacy of DTaP vaccines in infants [Table 1.1][14-20,217-220]. Comparisons between the studies are not straightforward as they varied in the type of vaccine, dosing schedule, case definition and ascertainment, and laboratory detection method[212,221,222]. Multi-component aP vaccines containing 3-5 pertussis antigens have higher efficacy than single or 2 component aP vaccines. One or 2 component aP vaccines range in efficacy from 67% - 70% while vaccines containing 3 or more components have between 80% - 84% efficacy. Comparison of aP to wP vaccine efficacy is difficult due to the heterogeneity of wP vaccines and variable number of aP vaccine antigen components. Three and 5 component aP vaccines appear to be more efficacious than wP vaccines in preventing pertussis as defined by the WHO. A study by Olin et. al found the wP vaccines more efficacious than aP, although this study is an outlier compared to other studies[19]. According to the CDC, aP vaccines are generally more efficacious than wP vaccines[67]. The WHO states that the best aP vaccines are more efficacious than low-performing wP vaccines but are not as efficacious as the best wP vaccines[12]. After the first dose of pertussis there is 15-20% protection and this level increases with each dose thereafter in the series[72].

One study in the U.S. was conducted to measure the efficacy of an aP vaccine formulated for adolescents and adults[48]. The vaccine was 92% effective (95% CI: 32-99%). The confidence interval around this estimate is large as only 10 pertussis cases met the primary case definition. Prior studies looking at the

immunogenicity and reactogenicity of the aP vaccines found them to be effective and safe[49,223-227]. While no aP vaccine efficacy studies have been conducted in older adults, results from a randomized trial demonstrated the aP vaccine was immunogenic and safe[228].

8.2.2.3 Duration of Immunity

While the duration of immunity following aP vaccines was previously thought to be similar to wP vaccines[208], more recent immunology and efficacy data indicate those receiving aP have lower protection than those vaccinated with wP vaccines[15,81,108,109,111-113]. Outbreak data from Australia and the U.S. found that priming with wP vaccine was associated with lower pertussis incidence compared to priming with aP vaccine[108,109]. Complementary data show there was a reduced risk for pertussis among persons ever vaccinated with wP vaccine versus persons with a history of 5 aP vaccinations[112].

Most studies estimate the duration of protection to be 5-6 years[229-231]. Vaccine efficacy for aP vaccines in children decreases substantially after receipt of the 5th dose[111,232-234]; those whose last vaccine was given greater than 60 months prior had 71% vaccine efficacy compared to 95% efficacy for those who had received the vaccine between 1-2 years prior. In infants the effectiveness of 3 doses drops even more quickly; vaccine efficacy between 3 and 4 years declines to 59%[235]. Similar waning immunity occurs in adolescents after a booster dose of Tdap[236]. Adolescents who received wP vaccines in childhood had greater protection against pertussis than those who received aP vaccines[237]. A study of

the adult aP vaccine found antibody levels to PT, FHA, and Prn significantly decreased 3 years post-vaccination[238]. While FHA and Prn antibody levels were still higher than pre-vaccination, PT antibody levels approached pre-vaccination levels, indicating potential susceptibility to pertussis infection.

8.2.2.4 Safety

Acellular pertussis vaccines are associated with many of the same adverse events as wP vaccines however the frequency and severity of these adverse events are reduced in the aP formulation[212]. There are no known serious adverse events caused by the aP vaccine. The final 2 doses of aP-containing vaccines containing diphtheria and tetanus antigens are associated with increased adverse reactions compared to the first three doses.

8.2.3 Novel vaccine development

Work is underway to develop new pertussis vaccines that have greater efficacy than current aP vaccines while maintaining their excellent safety profile[239,240]. Theoretical candidates including adding or changing virulence factors in aP vaccines, modifying the adjuvant to improve the Th1 response, creating protein conjugate vaccines, using outer membrane vesicles (nanoparticles) to deliver a multitude of pertussis antigens, and reducing the level of endotoxin in wP vaccines[239,241-248]. Phase 1 studies are underway for a live attenuated pertussis vaccine[241].

8.2.4 Vaccination Schedule Recommendations

8.2.4.1 U.S. Schedule

The pertussis vaccine is administered intramuscularly in the thigh for infants or deltoid for adolescents and adults in 0.5 mL volume[12]. In the U.S. five doses comprise the primary vaccination sequence (DTaP) to be given at 2, 4, 6 months, between 15-18 months, and a last dose between 4-6 years[249]. An adolescent booster (Tdap) should be given between years 11-12.

Adults should receive one dose of Tdap as a booster shot[250]. Pregnant women should receive one dose of Tdap vaccine during each pregnancy regardless of prior vaccination history[251]. Ideal timing for vaccination during pregnancy is 27 to 36 weeks gestation for optimal transport of antibodies to infants.

8.2.4.2 Expanded Programme on Immunization Schedule

Primary vaccination series schedules vary by country (e.g. 2, 4, 6 months or 2, 3, 4 months or 3, 5, 12 months)[12]. The WHO recommends a three dose primary series at 6 weeks, 10-14 weeks, and 14-18 weeks. All three doses should be administered by 6 months of age. An additional booster dose is recommended at age 2 years. The WHO does not recommend pertussis vaccines for adolescents and adults.

8.2.5 Immunization Programs

8.2.5.1 Global

The WHO goal for pertussis vaccination is >90% coverage of 3 doses in infants[12]. While both aP and wP vaccines are generally safe and efficacious, the aP vaccine is considerably more expensive and therefore its use has been limited to middle and upper income countries. Whole cell pertussis vaccines are standard in low-income countries. In 2001 a collaboration of global pertussis experts formed The Global Pertussis Initiative to recommend pertussis vaccination strategies and identify research gaps[55,252]. The panel recommended that countries with high infant vaccination coverage add a booster dose at 4-6 years, a booster to adolescents, and to target adult groups. Universal adult vaccination was recommended in countries with resources to support adult immunization programs.

SAGE recently provided updated guidance on the choice of pertussis vaccines for countries[93]. Based on current evidence aP vaccines are less efficacious and have more rapidly waning immunity compared to wP vaccines. In settings where less than 5 doses of pertussis vaccine are given wP vaccines should continue to be used for the primary vaccination series. The use of wP vaccines is likely a more effective strategy to protect infants than aP vaccines when booster doses are not readily available. Countries should only consider switching to aP vaccines if they have adequate resources and are prepared to provide several booster doses to reduce transmission to young children.

8.2.5.2 Nepal

Nepal started its National Immunization Programme (NIP) in 1979 with the Bacillus Calmette-Guérin (BCG) and DPT vaccines[253]. Nepal's current schedule includes BCG, oral polio vaccine (OPV), DPT-HepB-Hib, and measles for children, Japanese encephalitis in districts with high risk of transmission, and tetanus toxoid (TT) for pregnant women, all of which are provided without cost at government facilities. Combination measles-rubella (MR) vaccines were initially introduced in June 2013. Diphtheria, Pertussis, Tetanus (DPT) vaccine coverage has increased substantially in Nepal with only 54% of children fully vaccinated by 12-23 months of age in 1995 compared to 90% in 2012[253]. The most recent data from 2011 give DPT1, DPT2, and DPT3 coverage at 96%, 95%, and 92%, respectively[254]. In the central terai region, where Sarlahi District is located, 96%, 92%, and 87% of children have received one, two, and three doses, respectively, of DPT by ages 12-23 months. While the coverage is high many receive vaccines on a delayed schedule and not by the 6-month deadline recommended by the WHO. As a result the under-immunized are vulnerable to disease when the morbidity and mortality consequences are greatest. In Nepal, male children and those who have lower birth order are more likely to be fully vaccinated than female children and those with a higher birth order[254].

Nepal considers its NIP a high priority and the country is on track to achieve MDG 4 on child mortality reduction[255,256]. Nepal's immunization program is supported mainly through GAVI (46%), Nepal's government (45%), and the WHO (9%)[147]. The United Nations Children's Fund (UNICEF) and Japanese Interna-

tional Cooperation Agency (JICA) also contribute. The NIP has eight objectives[147,255]:

1. Achieve and sustain 90% coverage of 3 DPT doses by 2008 and all antigens in all districts by 2010;
2. Maintain polio free status;
3. Sustain maternal and neonatal tetanus elimination status;
4. Initiate measles elimination initiatives from 2010;
5. Expand vaccine preventable diseases (VPD) surveillance;
6. Accelerate control of other VPD through introduction of new vaccines;
7. Improve and sustain immunization quality;
8. Expand immunization service beyond infancy.

The recommended schedule for infant DPT vaccine in Nepal is 6, 10, and 14 weeks. For pregnant women 2 doses of tetanus toxoid are recommended. Pertussis is not currently recommended as a booster immunization for adolescents and adults, including pregnant women.

8.2.5.3 Vaccination delay

Children who are unimmunized or under immunized are at increased risk for pertussis and pertussis hospitalization compared to their more fully immunized peers[27,118,158-160]. National immunization coverage figures do not fully capture the excess pertussis risk period attributable to delays in vaccination. Further some countries' official reporting may overestimate the coverage in part to reach

donor targets such as GAVI's immunization services support (ISS)[257]. National-level reporting may mask within-country variation in vaccination timeliness[258].

Globally, WHO and UNICEF use officially reported data and sample survey data to measure DPT coverage of children 12-23 months[257]. As result if there are substantial delays in vaccination, but DPT vaccines are complete by age 2, a child is still considered as vaccinated on schedule. In the U.S. and elsewhere standard national reporting statistics obscure delays when infants are at risk for vaccine-preventable diseases[27,258-260]. For example, Japan's reported DPT3 coverage was 98% in 2013, however data from a representative city in Japan showed less than 50% DPT coverage by age 12 months[27]. In the U.S. a study found almost half of children with some delay in receiving a DTaP vaccine dose and 16% under vaccinated for more than 6 months in the first two years[259], while national DPT3 coverage in 2013 was high at 94%. A study examining the timing of vaccination in low and middle income countries, based on surveys and imputed data, found median DPT1 coverage at 6 months was 82% (95% CI: 67-89%) and DPT3 was 36% (95% CI: 23-54%)[258].

In the U.S. vaccination delay is associated with a mother who is unmarried, less educated, non-Hispanic black, and the use of public vaccination providers[259]. Reasons for vaccination delay in low and middle-income countries include poor immunization supply, access to health services, and family characteristics[260-262]. Parents may also be hesitant to vaccinate or not view the costs involved with vaccination worth the benefit. A longitudinal study in Ghana reported that while DPT3 coverage was 95% at 12 months, only 10% of infants were vac-

cinated within 1 week of the scheduled time (14 weeks); median delay for DPT3 was 4.0 weeks[261]. Infants who were poorer, had less educated mothers, and lived in rural versus urban areas were significantly more likely to be delayed in vaccination compare to urban infants whose mothers were educated and in a higher income grouping. A study of 31 low and middle income countries also found that children in poorer families and families with more than one child were at increased risk for vaccination delay[260].

8.2.6 Infant Immunization Strategies

The majority of pertussis deaths occur prior to 3 months of age, before an infant is able to be fully vaccinated[121]. Multiple strategies are available to prevent pertussis infection in this youngest age group.

8.2.6.1 Adolescent boosters

While an analysis of the U.S. adolescent Tdap program found it was associated with a decrease in adolescent pertussis, the authors found no indirect benefit to infants[263]. The cost-effectiveness of this strategy to prevent pertussis in infants is unclear[264].

8.2.6.2 Cocooning

A potential strategy to protect infants is to vaccinate close-contacts, including post-partum mothers in an effort to cocoon the infant from disease exposure. A systematic review found, unsurprisingly, that mothers followed by fathers, are most likely to transfer disease to their infants and therefore should be priori-

tized for the cocooning strategy[148]. Cocooning was recommend by the ACIP in 2006, however uptake has been poor[83]. High vaccination costs, feasibility of vaccinating all contacts, and lack of demonstrated efficacy continue to hamper efforts[265].

The cocooning strategy is highly resources intensive. A Canadian study found the number needed to vaccinate to prevent one infant death was 1 million parents and to prevent 1 ICU admission was 100,000 parents[266], similar to findings from other studies[267]. Cocooning has had most success when family members are vaccinated during the hospital delivery stay rather than at a later time point in the community[268,269].

Cocooning is among the most expensive strategies to protect infants[270]. Cost-effectiveness studies show mixed results on the effectiveness of post-partum vaccination[264,271,272], although cost-effectiveness analysis is highly dependent on the assumed disease incidence. Post-partum vaccination was less cost-effective and efficacious than vaccination during pregnancy[273].

While one modeling study found a modest effect of cocooning[146], data from implementation studies have shown no effect of vaccinating mothers in the post-partum period in reducing pertussis illness in their infants[265,274]. If mothers are vaccinated in the immediate post-partum period there is also a lag period of approximately 2 weeks until they are immune when the infant is still at risk[275].

8.2.6.3 Immunization at Birth

Another strategy is immunization with pertussis vaccine at or near the time of birth. As the burden of disease is large in the youngest infants, initial studies of pertussis vaccine examined the earliest possible timing of immunization that would elicit an infant immune response[276,277]. Researchers found that wP immunization in early infancy was not effective; between 2 and 7 months was found to be an ideal time for pertussis vaccination[121,278]. Recent studies with the aP vaccine have re-examined this concept with mixed results; some studies indicate it primes and stimulates the immune system for subsequent aP immunizations[279-281] while others demonstrate birth immunization inhibits future aP immunization[282]. A modeling study showed moving the age of vaccination up 2 weeks in the U.S. would reduce infant pertussis hospitalizations by 9%[122]. More studies are needed to further elucidate the benefits/risks of this strategy. Even if this strategy is successful in boosting infants' immune response, infants will still remain at risk in the window between birth and the stimulation of the immune system against pertussis.

Two reasons for poor immune response at birth are competing maternal antibodies and an immature infant immune system[278,283]. An explanation for maternal antibody inhibition is that the maternal antibodies mask pertussis vaccine epitopes, interfering with antigen binding by infant B cells[284]. One recent study comparing children born to HIV positive and negative mothers found that HIV-exposed but uninfected children born to HIV positive mothers had lower levels of maternally-derived pertussis antibodies at birth, but a subsequently higher re-

sponse to wP vaccine[284]. Maternal antibody vaccine interference appears to be dependent on vaccine type (aP versus wP). Some studies have demonstrated that infants born with high cord blood pertussis antibody titers have reduced response to wP vaccination; in contrast, circulating maternal antibodies provide little to no interference with infant immune response to the standard aP vaccine schedule[278,279,285-290].

8.2.6.4 Maternal Immunization

8.2.6.4.1 Recommendations

The most favorable strategy to protect infants is to immunize women during pregnancy with an aP vaccine to boost maternal pertussis antibody levels prior to birth; passive transfer of antibodies through the placenta will confer protection until the infant is able to be vaccinated himself[291]. Acceptance and recommendation of maternal immunization has rapidly evolved in recent years with increasing pertussis infant incidence. In 2005 the U.S. ACIP recommended the vaccine to post-partum mothers[83]. In June 2011 the ACIP recommended pertussis vaccine during pregnancy for women who had not previously received Tdap due to the increasing number of infant deaths, especially in those less than two months[292]. Subsequently in October 2012 the ACIP revised its recommendation to support a Tdap vaccine in each pregnancy, regardless of prior immunization status. While the vaccine can be given at any time during pregnancy, optimal timing is between 27 to 36 weeks gestation[251]. The United Kingdom also implemented a maternal vaccination program in response to infant pertussis resurgence[293,294].

Maternal vaccination is a promising strategy today as there is a licensed aP vaccine recommended for adults and there is a licensed aP vaccine for children, which is not inhibited by high levels of maternal antibodies[291,295-297]. While the safety of Tdap in pregnancy warrants further investigation, initial studies raised no safety concerns[293,298]. Pregnant women already interact frequently with health care workers for antenatal care visits. Acellular pertussis vaccines could be given in combination with other vaccines routinely given in pregnancy (U.S. – influenza, tetanus, and diphtheria; Nepal – tetanus). An advantage of maternal immunization compared to infant or postpartum immunization is that protection would be available at birth instead of a lag while the infant or mother develops her own immune response[275]. In addition, it would benefit both the mother and child. Providing Tdap in pregnancy versus in the post-partum period is also more cost effective with an estimated $414,523 versus $1,172,825 per quality-adjusted life year saved[273].

8.2.6.4.2 History

Maternal immunization during pregnancy as a strategy to protect infants from pertussis was investigated as early as the 1930s[276]. In early studies, infant antibody titers were between 50-100% of their vaccinated mothers[276,299-302]. One randomized trial showed infants of pertussis immunized mothers had almost three times higher antibody levels than infants of mothers in the control arm (unvaccinated)[300]. In another trial, serum from infants of immunized mothers was given to mice that were then challenged with virulent pertussis; the serum was found to be quite protective in the mouse model[301]. While no safety concerns

were raised, maternal vaccination was not pursued extensively due to concern that maternal antibodies would interfere with subsequent infant immune response to wP vaccine[123]. In addition, the previous research was conducted with wP vaccine, which is not currently recommended for adults in any setting due to increased reactogenicity.

8.2.6.4.3 Maternal Antibody

Maternal antibodies may protect infants from morbidity or prevent severe morbidity although this depends on the level of maternal antibodies, the efficacy of transfer and the rate of antibody decay in the infant[121]. While high levels of pertussis-associated antibodies are correlated with protection from disease, a specific cutoff has not been established[74,76-79]. Therefore the proportion of infants born with protective levels of antibodies is unknown. Historical U.S. data (1938-1940) indicate potential protection from maternal antibodies; infant pertussis deaths in the first month of life were one-third of that in the 2nd and 3rd months[303]. More recent data shows no difference in the rates of death between the first and 3rd months of life[24]. One explanation for this is lower levels of endemic pertussis in the vaccine era led to lower pertussis exposure in pregnant women. Lower levels of pertussis exposure may lead to lower levels of maternal antibodies. Higher exposure due to circulating pertussis in previous eras may have boosted maternal antibody levels high enough to provide protection to their infants. Support for this hypothesis comes from pertussis serological data. While not directly comparable, pertussis antibodies were detected in 30-50% of women in the pre-vaccine era[304,305], while a lower percentage of contemporary women harbor high

levels of pertussis antibodies[123,279,286,306]. A recent study of mother-infant pairs found only 21% of mothers had PT antibody >5 EU[307].

Pertussis vaccinated women have a rapid response with IgG titers that peak at day 14 and IgA titers peaking at day 10[275]. This potentially indicates that maternal vaccination may protect transplacentally (IgG) and through breastmilk (primarily IgA). Mothers with a chronic disease(s) were found to have lower PT antibodies than mothers with no chronic health condition[306]. However, HIV infection was not shown to be associated with lower PT antibodies compared to those mothers who are HIV negative[284].

8.2.6.4.4 Infant Antibody

Infants whose mothers have higher antibody levels due to recent infection or vaccination have higher antibody levels than infants of unexposed or unvaccinated mothers[307-309]. Pre-term infants have lower antibody concentrations compared to term infants[310-312]. Uninfected infants born to HIV positive women have lower PT antibody compared to infants born to HIV negative women[284].

Pregnancy history, occupation, education, ethnicity, marital status and number of household members have previously been found to have no association with infant PT antibody levels[307]. The effect of maternal age on infant antibody levels is mixed with some studies showing an association with increased maternal age and higher PT antibody[284,307].

8.2.6.4.5 Maternal to Infant Antibody Transfer

Immunoglobulin G is transferred from mother to fetus through the placenta during pregnancy[121,285]. The exact mechanism of transport is unknown. Briefly

placental Fc receptors bind maternal IgG resulting in active transport of antibodies to the fetus[313,314]. The IgG1 subclass is transferred with greater efficiency than other subclasses or IgM, IgA, and IgE[315]. Maternal antibody transfer begins at approximately 16 weeks gestation and increases until time of delivery[316]. While this is an active process the transfer efficacy has been shown to range from 20% to 200% for various antibodies[317]. Research from the 1940s measured low efficiency of maternal to infant pertussis IgG transport with only 2-12% of infants having higher antibody levels than their mothers at birth[121]. However, a strong correlation between mother and infant pairs was found. Unsurprisingly, mothers who previously contracted pertussis or were immunized with wP vaccine during pregnancy gave birth to infants with the highest pertussis antibody titers.

Multiple recent studies have examined the association of pertussis antibodies between mother and infant pairs as a proxy for efficiency of antibody transfer and have found a strong association [Table 1.2]. Transfer is active from mother to child with the ratio of infant to maternal antibody concentration (term infants) at 107-169% for PT, 120-178% for FHA, 112-157% for FIM and 120-131% for Prn[123,284,307,309,311,312,316,318,319].

Transplacental antibody transfer efficiency in general (non-pertussis-specific) has not been found to be associated with parity, maternal age, height or weight[310,318]. Transport is associated with maternal ethnicity, vaccination and health status[310]. Pre-term infants have lower antibody concentrations compared to term infants[310-312]. Moreover, protective antibodies persist for shorter duration in pre-term infants. The lack of protective antibodies combined with an immature

immune system puts these pre-term infants at increased risk of contracting pertussis.

For mothers who receive Tdap in pregnancy, antibodies between mothers and infants are highly correlated[309]. Newborns of vaccinated mothers had higher concentrations of pertussis antibodies and were more likely to have serological protection from pertussis compared to unvaccinated mothers[309,320]. The timing of maternal vaccination is important; immunization pre-partum and in early pregnancy provides less protection to infants than vaccination in the third trimester[308].

Maternal IgG antibodies to PT and FHA transferred to infants have an ap-

TABLE 1.2

Pertussis Antibody in Unimmunized Mothers and Infants

Antigen	Number of Samples Tested		Infant: mother	Correlation	Geometric Mean Concentation (GMC)				Reference
					Mother		Infant		
	Mother	Infant							
PT	196	196	1.64	0.8 (?)	9.9	8.6 - 11.3 (95% CI)	16.2	14.2 - 18.3 (95% CI)	de Voer (2009)
	101	103		0.98 (P[C])	4.4	2.6 (SD[E])	5.6	3.0 (SD)	Gonik (2005)
	64	61	1.69		2.4	1.9 - 3.1 (95% CI)	4.1	3 - 5.5 (95% CI)	Healy (2004)
	39[A]	42[A]	1.30		5.2	3.5 - 7.8 (95% CI)	6.2	4.1 - 9.4 (95% CI)	van den Berg (2010)
	88[B]	96[B]	0.64		7.3	6.0 - 9.9 (95% CI)	5.4	4.2 - 6.9 (95% CI)	
	58	54	1.51	0.89 (S[D])	23.6	12.9 - 54.7 (IQR[F])	36.1	20.4 - 76.3 (IQR)	Jones (2011)
	52	52		0.16 (P)			11.0	1.8 (SEM[G])	Gall (2011)
	99	96	1.56	0.71 (S)	18.7	15.3 - 22.8 (95% CI)	29.1	23.4 - 36.3 (95% CI)	Jones (2013)
	100[A]	100[A]	1.07	0.95 (P)	18.2	15.0 - 22.0 (95% CI)	19.5	15.7 - 24.1 (95% CI)	Erener Ercan (2013)
	100[B]	100[B]	0.68	0.80 (P)	19.4	15.5 - 24.3 (95% CI)	13.2	10.2 - 17 (95% CI)	
FHA	195	192	1.62	0.87 (?)	21.5	18.6 - 24.8 (95% CI)	34.8	30.1 - 40.1 (95% CI)	de Voer (2009)
	101	103		0.90 (P)	26.6	3.1 (SD)	10.2	3.2 (SD)	Gonik (2005)
	64	61	1.78		6.9	5 - 9.5 (95% CI)	12.3	8.8 - 17.3 (95% CI)	Healy (2004)
	39[A]	42[A]	1.37		10.2	7.3 - 14.1 (95% CI)	14.1	10.3 - 19.4 (95% CI)	van den Berg (2010)
	88[B]	96[B]	0.65		15.1	12.0 - 19.0 (95% CI)	9.8	7.9 - 12.2 (95% CI)	
	52	52		0.17 (P)			26.8	4.0 (SEM)	Gall (2011)
	100[A]	100[A]	1.20	0.95 (P)	16.0	13.1 - 19.5 (95% CI)	19.2	15.6 - 23.6 (95% CI)	Erener Ercan (2013)
	100[B]	100[B]	0.72	0.86 (P)	20.3	16.6 - 24.8 (95% CI)	14.6	11.6 - 18.2 (95% CI)	
PRN	196	195	1.31	0.84 (?)	13.5	11.7 - 15.6 (95% CI)	17.7	15.2 - 20.5 (95% CI)	de Voer (2009)
	101	103		0.96 (P)	12.3	2.9 (SD)	10.2	3.2 (SD)	Gonik (2005)
	39[A]	42[A]	1.20		4.1	2.8 - 6.1 (95% CI)	4.7	3.2 - 6.8 (95% CI)	van den Berg (2010)
	88[B]	96[B]	0.66		4.9	3.7 - 6.6 (95% CI)	3.2	2.4 - 4.3 (95% CI)	
	52	52		0.97 (P)			26.8	4.0 (SEM)	Gall (2011)
FIM	64	61	1.57		13	9. 2 - 18.5 (95% CI)	20.4	14.0 - 29.6 (95% CI)	Healy (2004)
	39[A]	42[A]	1.12		10.4	6.4 - 17.0 (95% CI)	10.1	6.8 - 14.8 (95% CI)	van den Berg (2010)
	88[B]	96[B]	0.69		12.3	9.0 - 17.0 (95% CI)	8.7	6.5 - 11.7 (95% CI)	
	52	52		0.29 (P)			82.8	14.6 (SEM)	Gall (2011)

[A] Term Infants; [B] Preterm Infantsl [C] Pearsons correlation; [D] Spearmans correlation;[E] standard deviation, [F] interquartile range; [G] standard error of the mean

proximately 5-week half-life in the infant. In unvaccinated mothers detectable pertussis antibodies persist for at most 2-4 months and in vaccinated mothers this extends to 6 months[123,278,285,299]. High maternal antibodies from Tdap in pregnancy were associated with a lower infant response to the primary vaccination series; however, responses equalized after the booster dose[320].

8.2.6.4.6 Efficacy of maternal antibodies in protecting infants

Animal models support the hypothesis that higher pertussis antibody titers confer protection against pertussis challenge[321-323]. A case control study comparing infants <6 months who contracted pertussis to controls found lower pertussis antibody levels at birth in the case compared to control infants although the differences did not reach statistical significance[289].

Until recently, no studies had measured the efficacy of maternal aP vaccination on protecting infants from pertussis[121]. Two randomized controlled trials of aP vaccine in pregnancy are ongoing in the U.S. and Canada to assess its safety, efficacy in protecting infants from pertussis, and its effect on infants' subsequent response to the primary pertussis immunization series[324,325]. Early results from the U.S. clinical trial found pertussis vaccination in pregnancy to be safe, immunogenic and not blunt the immune response to infant pertussis immunization[326].

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CHAPTER TWO

Methods

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Section 1 - Aims

The objective was to prospectively characterize pertussis epidemiology in rural Nepal including pertussis vaccination coverage, infant pertussis incidence, and pertussis antibody transfer between mothers and their infants. The rationale for this study was that without a well-defined burden of pertussis disease it is difficult to demonstrate a need for public health interventions. Such knowledge is critical to justify further pertussis research and potential adoption of maternal immunization in developing countries to reduce disease in infants too young to be directly immunized.

Subsection 1.1 – Primary Aims

1. Estimate the timing of pertussis vaccination in infants 0 to 6 months in Nepal.
2. Estimate the incidence of *B. pertussis* infection in infants 0 to 6 months in Nepal.
3. Estimate the ratio of infant to maternal pertussis toxin (PT) antibody at birth in Nepal.

Subsection 1.2 – Secondary Aims

1. Identify infant, maternal, and household characteristics associated with pertussis vaccination delay.

2. Estimate the incidence of *B. parapertussis* infection in infants 0 to 6 months in Nepal.
3. Describe the clinical, infant, maternal, and household characteristics associated with infant pertussis.
4. Identify infant, maternal, and household characteristics associated with infant PT antibody titers.
5. Identify maternal and household characteristics associated with maternal PT antibody titers.
6. Identify infant, maternal, and household characteristics associated with PT antibody transfer ratio.

Section 2 – Study Design

This prospective cohort study was based in Sarlahi District, Nepal, a densely populated, low-lying area near the Indian border. The study population included 3,690 pregnant women and their live born infants followed from delivery through 6 months post-partum. The study was nested within a community-based randomized controlled trial to test the efficacy of influenza vaccine in pregnancy for protecting mothers and infants from influenza[1].

Subsection 2.1 – Setting

2.1.1 Nepal

The study was conducted in Sarlahi District located in the central terai (low lying plains) region of the Federal Democratic Republic of Nepal[2]. As a recently

democratic multiparty republic, its first president was elected in 2008. Topographically Nepal is portioned into three unique ecological zones: mountain, hill, and terai [Figure 2.1][2]. The terai region is home to half of Nepal's population and is relatively fertile and flat compared to the hill and mountain regions. The hill region, which includes Kathmandu, the capital, holds 43% of the population. Only a small fraction of Nepalese live in the mountain region due to its harsh environment and inaccessibility; the elevation in the mountain region ranges from 4,877 to over 8,000 meters. In addition to the geographic divisions there are administrative divisions with 5 development regions (Eastern, Central, Western, Mid-Western, and Far-Western Nepal is further subdivided into 75 administrative districts within which are village development committees (VDCs), each of which contains 9 wards.

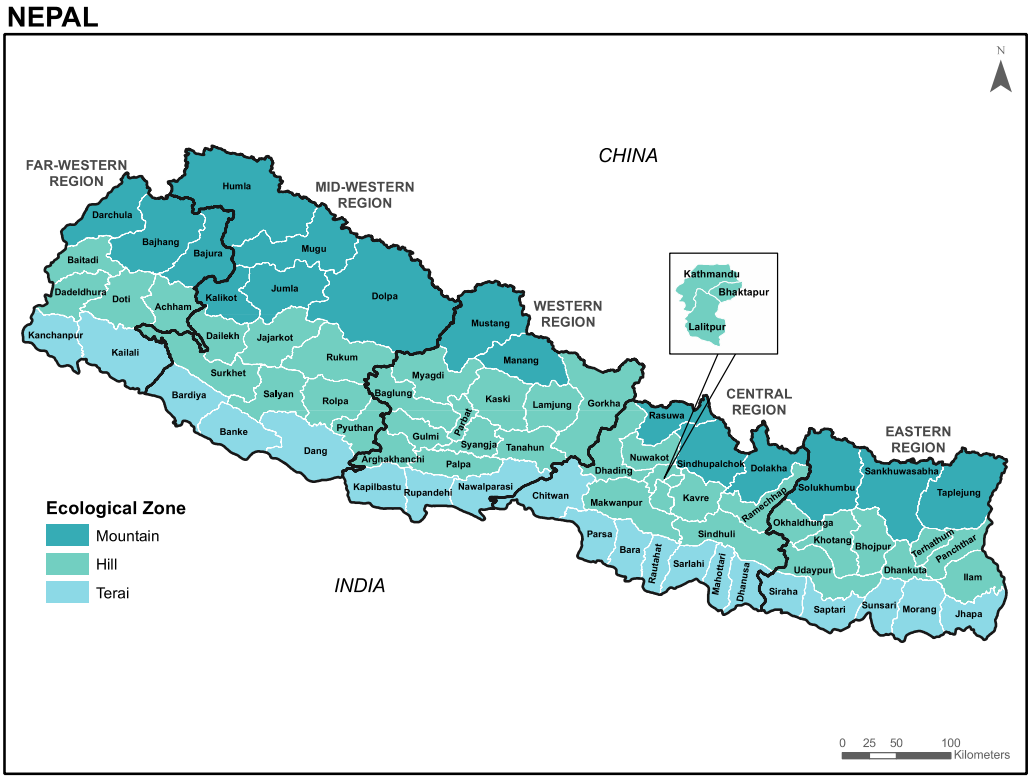

FIGURE 2.1 – NEPAL COUNTRY MAP

The majority (83%) of the estimated 26.6 million Nepalese live in rural areas[2]. One hundred three ethnic/caste groups exist in Nepal including Chhetri, Brahmins, Magar, Tharu, Tamang, and Newar. Over 92 languages are spoken, however Nepali is the official language. The majority of Nepalese practice Hinduism and a smaller fraction practice Buddhism, Islam, and Kirat. Life expectancy is approximately 69 and 67 for females and males, respectively[3]. The total fertility rate in 2012 was 2.4.

Nepal is one of the poorest countries in the world with approximately one-fourth of the population living below the poverty line[2]. Political instability, internal conflict and poor policy and planning have contributed to lack of development. The majority of the population's occupation involves agriculture. Remittances are a source of income for over half the population.

2.1.2 Sarlahi District

Approximately 636,000 people live in Sarlahi in predominately rural, farming communities[4,5]. Two major ethnic groups comprise the majority of the population[5]. The Madeshis, whose language is Maithili are mostly lower castes and the Pahadis are mostly higher castes and speak Nepali. Pahadis currently residing in Sarlahi migrated down from the hill region 30-40 years ago when malaria was first eradicated from the area; they are of Tibeto-Burmese origin. The Madeshi population is of north Indian ethnicity. The total fertility rate in the terai region in 2011 was 2.5 and the median age of first birth was 19.5 years[2]. In the central terai the proportion of women delivering has increased in recent years

with an estimated 32% of women giving birth in a health facility. In the terai region under-five mortality was 62 deaths per 1,000 live births and infant mortality was 53 deaths per 1,000 live births[2]. Child nutrition is an important problem in central terai with 41% of children stunted, 10% wasted, and 32% underweight.

The location of the study was the field site of the Nepal Nutrition Intervention Project (NNIPS). The site was established in 1989 for a large, community-based Vitamin A supplementation intervention trial[6]. Since then, Johns Hopkins Bloomberg School of Public Health researchers have worked continuously in the district performing other community-based randomized control trials and follow-up studies. Sarlahi contains 99 VDCs and the maternal influenza vaccination study site included VDCs in northern Sarlahi (orange) [Figure 2.2]. The VDCs include Raniganj, Jabdi, Lalbandi, Netraganj, Sasapur, Hariaun, Dhungrekhola, Karmaiya, and Ghurkauli[1]. Two other community trials were concurrently conducted in neighboring VDCs, however there was no overlap in study areas.

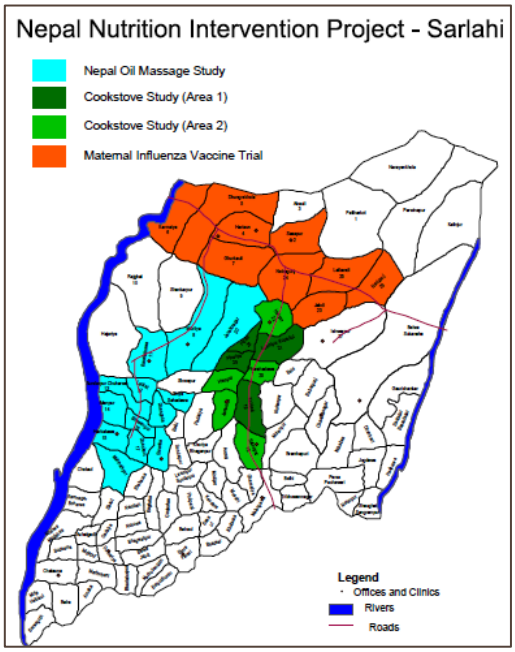

FIGURE 2.2 - NNIPS STUDY AREAS IN SARLAHI DISTRICT

Subsection 2.2 – Parent Maternal Immunization Trial

2.2.1 Aims

The parent study was a community-based randomized trial to test the efficacy of maternal administration of influenza vaccine for mothers and their children. Individual mothers were randomized to receive either an influenza vaccine or a placebo. The three co-primary aims were:

1. To compare the incidence of laboratory confirmed influenza illness episodes among newborn infants (through 6 months of age) born to women randomized to receive either influenza vaccine or control during pregnancy.
2. To compare the incidence of low birthweight (<2500 grams) of newborn infants born to women randomized to receive either influenza vaccine or control during pregnancy.
3. To compare the incidence of influenza-like illness (ILI) episodes among pregnant women (through 6 months postpartum) in women randomized to receive either influenza vaccine or control during pregnancy.

2.2.2 Study Population

The study population included all pregnant women identified with gestational age between 17 and 34 weeks during a 24-month period (2 12-month cohorts) in 9 VDCs of Sarlahi District, Nepal[1]. At the start of the trial, prevalent pregnancies were identified through a survey census of all households in the catchment area

and women between 17 and 34 weeks gestation were randomized to receive influenza vaccine or a saline placebo. Thereafter, field workers visited homes of married women of reproductive age every 5 weeks to monitor for incident pregnancies. All pregnant women meeting the eligibility requirements and providing informed consent were enrolled. Women were excluded from the study for the following reasons:

- Planned to give birth outside of the study area
- Already vaccinated with current influenza vaccine
- Already enrolled in the same trial during previous pregnancy
- Known allergies to any component of the vaccine (e.g. eggs)
- Infant delivery occurred <2 weeks after receipt of immunization (influenza or placebo)
- Did not provide consent to participate in the trial

All women meeting the eligibility requirements were recruited to enroll in the trial. The study was described to prospective participants. If a woman agreed to enroll her consent to participate in the trial was obtained. Following consent, her information was recorded in a pregnancy tracking roster. All participants received ancillary benefits, which included a 90-day supply of iron-folic acid tablets, deworming medicine (single dose of albendazole), clean birthing kit, chlorhexidine ointment for umbilical cord care, a tetanus toxoid (TT) vaccine, if indicated, and health education messages, in addition to antenatal services according to the local standard of care.

2.2.3 Intervention

The trial intervention was a trivalent inactivated influenza vaccine, licensed for use in North America, Europe, or Australia. The vaccine was updated throughout the trial as new formulations with the current season's strains became available.

The women assigned to the control group were given a placebo (saline injection). The justification for a placebo arm was twofold. First, the influenza vaccine is not currently recommended in Nepal and the Ministry of Health is highly interested in the results of the study to help inform future immunization policy. Second, no placebo-controlled studies have been conducted to examine the effect of maternal influenza vaccination on respiratory morbidity in early infancy. By including a placebo arm, this study will be able to answer important questions regarding the efficacy of maternal vaccination on protecting mothers and their infants.

2.2.4 Randomization

Pregnant women were individually randomized at enrollment to receive either a placebo or influenza vaccine. Randomization of women, in block size 8, was stratified by VDC and gestational age at time of vaccination (17-25 and 26-34 weeks) to ensure balanced distribution of immunization assignment in each geographic area and by gestational age. In the first cohort (April 25, 2011 – April 24, 2012) women were vaccinated as soon as possible after 17 weeks gestation. In the second cohort, in addition to randomization stratified by VDC and the two

previously noted gestational age periods, women were randomized to the timing of immunization between 17 and 34 weeks gestation to ensure an equal proportion of women vaccinated at different times during pregnancy rather than at 17 weeks as in cohort 1. At study headquarters a random number generator created individual randomization assignments to which investigators and study workers were blinded to treatment group. However, since the influenza vaccine and placebo syringes looked different, the vaccinators were not blinded to treatment group. Vaccinations were conducted with individual women alone in a closed room with the vaccinator to avoid unblinding of other study staff. Study outcomes were assessed by study staff who were not vaccinators. Four treatment codes were created (A, B, C, and D); two of these corresponded to placebo doses and two of these corresponded to influenza doses. Vaccinations for cohort 1 commenced April 25, 2011 and ended April 24, 2012. For cohort 2, vaccinations commenced April 25 2012, enrollment of pregnancies ended April 24, 2013 and the last vaccination was administered September 9, 2013. This lengthened vaccination period was due to the randomized timing of vaccinations in cohort 2.

2.2.5 Sample Size

The sample size for the larger trial was based on the necessary power for each of the three primary outcomes and was adjusted for multiple outcomes (to produce an overall type I error across the 3 aims of 5%). The calculated necessary sample size was 1,850 mothers and their live born infants for each annual cohort.

2.2.6 Data collection

2.2.6.1 Interview and measurement data

Data were collected at several periods during the study:

- A house-to-house census was conducted at baseline to identify all married women of reproductive age (15-40 years). Informed consent was obtained from eligible households and individual consent from women of reproductive age. Following consent, information was collected on household structure, socioeconomic status, and demographic information.
- Women of reproductive age who identified themselves as being pregnant during the baseline were enrolled and vaccinated if they were between 17 and 34 weeks gestation. Those who were less than 17 weeks were scheduled for vaccination once they reached 17 weeks. Following the baseline survey, a female health worker visited each household every 5 weeks to test for incident pregnancies. If a woman has not had a menstrual period within the preceding 5 weeks, a urine-based pregnancy test and date of last menstruation was obtained. If a woman had a positive pregnancy test, she was invited to participate in the study and individually consented and enrolled.
- Once enrolled, the following information was collected: mother's age, pregnancy history, medical history, date of last menstrual period, anthropometric measurements (weight and height), and expected delivery loca-

tion. The date of last menstrual period was used to estimate gestational age.

- As soon as possible after delivery the mother and child were visited at the household to collect information on the birth. This information included the delivery process, newborn care practices, delivery complications, infant's weight, length, head circumference and temperature.
- From enrollment in early pregnancy through six months post-partum a Flu Data Collector (FDC) visited enrolled households weekly. During the visit field staff asked the mothers about their and their infant's current health and health during the previous week. If a respiratory illness was identified (separate definitions for mothers and infants) then a mid-nasal swab was collected from one nare. In addition, other morbidities and whether care was sought (type of provider and treatment received) were assessed. Further, information was obtained on breastfeeding status and whether specific immunizations were received. Women were followed through 6 months post-partum regardless of the pregnancy outcome (miscarriage, stillbirth).
- A field supervisor visited households to conduct a verbal autopsy if a mother or infant died during the study. The supervisor used a revised WHO instrument to obtain information on illness and care-seeking behavior history. Two local physicians reviewed the report and independently assigned a cause of death. If there were discrepancies a consensus was formed, with the aid of a third physician if necessary.

2.2.6.2 Biological specimen collection

Nasal Swabs

Nasal swabs were collected during the weekly visit for mothers if on at least one day in the prior week a woman reported a fever plus at least one additional symptom: cough, sore throat, nasal congestion/runny nose or myalgia. Nasal swabs were collected for infants if the mothers reported any one of the following symptoms on at least one day in the prior week: fever, cough, wheeze, difficulty breathing, nasal congestion/runny nose or ear infection. On August 17, 2012 additional respiratory symptoms were added to infant weekly morbidity assessment to increase the sensitivity in detecting pertussis. The additional symptoms that would trigger an infant nasal swab were the following: apnea, cyanosis, whoop, and cough with vomiting.

To collect the nasal swab a sterile nasopharyngeal swab with a nylon-flocked tip was inserted into one nare approximately one-half the distance between the external nostril and the nasal bridge. Once inside, the swab was rotated 360° to obtain a mid-nasal specimen. The procedure lasts approximately 10-15 seconds, is not painful and does not cause side effects, although the process may be irritating. The swab was then removed and placed directly into 2.0 mL of Primestore (Longhorn Diagnostics, San Antonio, TX) buffer and swirled briefly before breaking the handle so that only the swab remained in the tube. Primestore is a transport and storage medium that lyses the cells, stabilizes the nucleic acid, and preserves the sample.

After transport to field headquarters 3 aliquots were made into 0.5mL cryo-tubes. Primestore aliquots were kept at room temperature or refrigerated until processing by the U.S. testing laboratory.

Blood Specimens

Maternal blood specimens were collected pre-vaccination (enrollment), one-week post-partum, and three months post-partum. Study nurses collected 5cc of blood by venipuncture in the woman's arm using standard sterile technique. The vials were placed on ice and transported to the field headquarters.

Approximately one month prior to delivery a sterile plastic cup was left with the women to collect umbilical cord blood after birth. The women or their birth attendant were requested to collect umbilical cord blood that drips from the cut end of the cord into the cup until at least 3-5cc of blood were collected. The women then covered the cup and called the study nurse to notify her of the birth. For mothers who delivered in health facilities in the area, facility staff obtained cord blood, which was then collected by study staff for normal processing. The blood was transported on ice to the central field-processing laboratory.

Once maternal and infant blood was sufficiently clotted it was centrifuged and the sera aliquoted into several vials. Serum samples were stored and shipped to the United States at -80° Celsius.

Subsection 2.3 – Pertussis Study

The pertussis prospective observational study was nested within the parent community immunization trial harnessing the extensive trial infrastructure to answer important questions regarding pertussis epidemiology in Nepal. The pertussis study added additional testing for specimens (nasal swabs and blood) already collected for the main study [Figure 2.3].

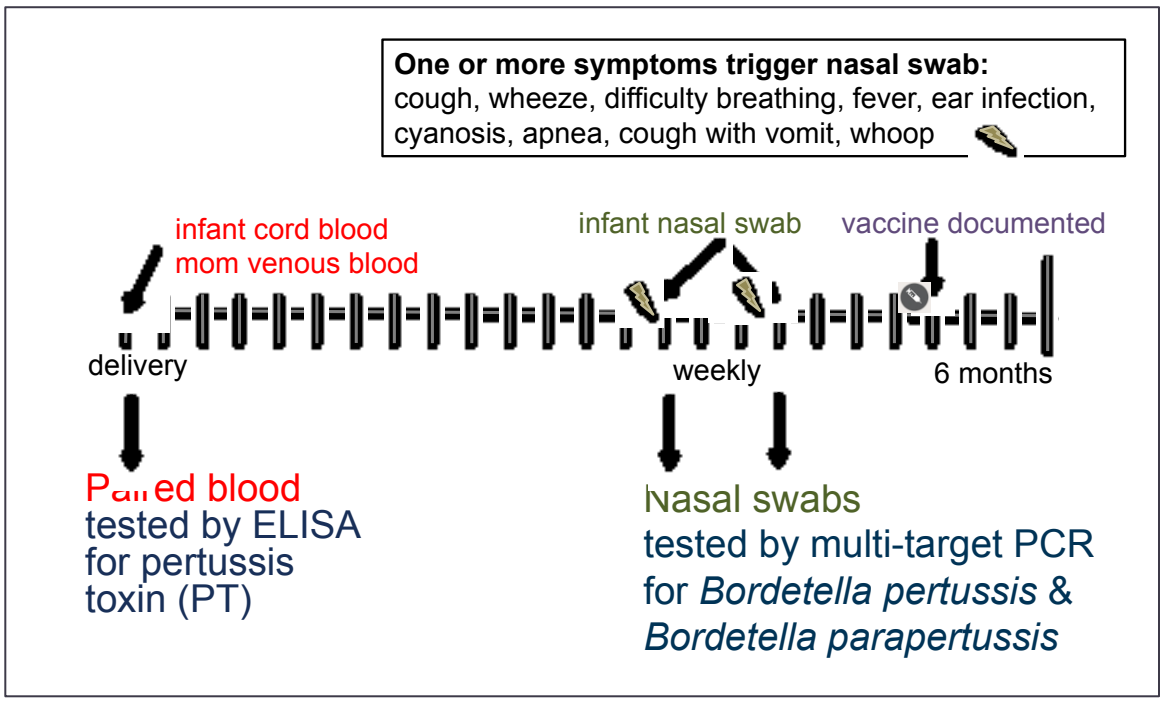

FIGURE 2.3 - PERTUSSIS KEY OUTCOMES DATA COLLECTION

2.3.1 Aim 1 – Estimate the timing of pertussis vaccination in infants 0 to 6 months in Nepal.

This aim characterized the timing of the primary DPT vaccination series (3 doses) in Nepal. DPT1, DPT2, and DPT3 coverage was estimated at 14 weeks (recommended age for completion of series) and 6 months (end of follow-up)

based on the weekly recall interviews with parents. Infants were included in this analysis if they were followed for a minimum of 14 weeks.

Pertussis vaccination in infancy is the primary pertussis prevention strategy globally. In low and middle-income countries the primary vaccination series is the only pertussis prevention method. Infants who are delayed in receiving their pertussis vaccine or who do not complete the entire series are at increased risk for pertussis compared to their more fully vaccinated peers[7-11]. Nepal immunization coverage estimates reported to the WHO do not capture vaccination delay in infancy when pertussis risk is greatest[11-14]. This aim was to improve the understanding of pertussis vaccination timing in the first 6 months in Nepal. Understanding what delays exist is important to better characterize how well the primary pertussis prevention strategy is implemented.

Secondary Aims

1.1 Identify infant, maternal, and household characteristics associated with pertussis vaccination delay.

The purpose of this secondary aim was to identify infant, maternal, and household factors associated with under immunization at 14 weeks and 6 months. Previous research in low and middle-income has shown risk factors for vaccination delay include poor immunization supply and access to health services, having mothers who were poorer, less educated, living in rural areas, and with more than one child[14-16]. A better understanding of Nepal-specific factors

contributing to vaccination delay can help programs focus on at-risk populations to increase on-time vaccination.

2.3.2 Aim 2 – Estimate the incidence of B. pertussis infection in infants 0 to 6 months in Nepal.

The incidence study was a prospective cohort study nested within the parent maternal influenza trial. Infants were included if they were followed for any length (0 to 180 days) during a 2 year-period from August 17, 2011 to August 16, 2013. The outcome of interest was symptomatic laboratory confirmed pertussis infection in infants. An infant was considered an incident pertussis case if he/she had a positive B. pertussis PCR test and met the following clinical criteria based on a weekly parental recall of symptoms:

Respiratory Illness Definitions

Year 1 - Experienced at least one of the following symptoms: cough, wheeze, difficulty breathing, fever or ear infection.

Year 2 – Any of the symptoms from year 1 or any of the following: cyanosis, apnea, cough with vomit, whooping cough/whoop.

New symptoms were added in year 2 to increase the sensitivity for detecting pertussis cases that might have been missed with the year 1 criteria. Additional positive tests were considered related to the primary positive finding and were not considered a re-infection. Therefore, once an infant tested positive for pertussis they were not considered at risk for a subsequent pertussis infection (6 months maximum). The primary purpose of the nasal swab collection was to test

for influenza and other viral infections. Pertussis-specific PCR testing was conducted on these same nasal swab specimens.

Only one population-based study on pertussis including infants from birth has been published to-date[17]. However, infants are known to have the highest risk of pertussis and to suffer the greatest pertussis morbidity and mortality from passive reporting and hospital-based studies[18]. Data from aP vaccine efficacy trials has provided some insight into the burden of pertussis in older infants. However, these studies had more stringent criteria for testing and less robust surveillance than our pertussis study[19]. Combined, these factors likely led to an underestimation of pertussis disease, especially atypical or mild pertussis that did not trigger testing or reporting. Further these studies monitored infants at a minimum > 3 months of age after at least 1 vaccination. Therefore the data did not capture the unvaccinated infant population <3 months for whom the disease burden is most severe. Moreover, only one aP vaccine trial was conducted in a low-income setting (Senegal) and there are no active surveillance data from Asia[17]. This study provides the first population-based active pertussis surveillance in infants in Asia.

Secondary Aims

2.1 Estimate the incidence of B. parapertussis infection in infants 0 to 6 months in Nepal

The same methodology used to ascertain *B. pertussis* incidence in Aim 2 was also used to estimate the incidence of the less common and serious *B. parapertussis*[20].

2.2 Describe the clinical, infant, maternal, and household characteristics associated with infant pertussis.

In additional to respiratory symptoms, infant, maternal and household characteristics, assessed through questionnaires conducted at baseline and follow-up points, were used to describe pertussis infants and non-pertussis infants [Table 2.1].

TABLE 2.1 - INFANT, MATERNAL, AND HOUSEHOLD CHARACTERISTICS

Infant, Maternal, and Household Characteristics		
Infant	**Maternal**	**Household**
Sex	Age	Number of household members
Age	Parity	Socioeconomic status
Birthweight	Breastfeeding status	
Gestational Age	Ethnicity	
Size for gestational age	Literacy	
DPT vaccination status		

Classical pertussis disease is most likely to occur in children. Pertussis is recognized as a severely under-diagnosed disease and therefore the true global burden is unknown[21]. Pertussis in young infants is often "atypical" and therefore may go unrecognized[22]. This study aimed to elucidate symptoms associated with pertussis illness in infants to help clinicians better recognize the disease, which may have a mild or non-traditional presentation, especially in young infants.

Previous literature has documented risk factors for reported pertussis in infants such as being female, younger, under-immunized, preterm, born to a younger mother and a member of an ethnic or racial minority group[23-25]. Our study aimed to add to knowledge of pertussis risk factors. First, the multitude of data collected on potential risk factors is highly comprehensive compared to the majority of studies with less comprehensive tracking of participants. Second, the study was community-based with less potential for bias in assessing risk factors compared to hospital-based or outbreak-based studies. Further, the majority of studies on pertussis risk factors have been conducted in high-income countries. This study adds to literature on the burden of disease in a typical rural Asian population for which we expect the disease burden to be highest.

2.3.3 Aim 3 – Estimate the ratio of infant to maternal pertussis toxin (PT) antibody at birth in Nepal.

The third aim was to estimate the ratio of infant to maternal pertussis antibodies for PT. The ratio is an approximation of the efficacy of maternal to infant antibody transfer. A convenience sample of mothers and infants, for whom blood samples for both could be obtained, were included. ELISA testing for PT antibodies was added to maternal and cord blood samples originally collected for influenza antibody testing. The study was a prospective cohort of mother-infants pairs.

Previous literature has documented that there is active transfer of pertussis-related antibodies from mother to infant[26-34]. However, none of these studies

was conducted in a low-income country setting with high prevalence of malnutrition and prematurity.

Secondary Aims

3.1 Identify infant, maternal, and household characteristics associated with infant PT antibody titers.

The purpose of this secondary aim was to identify factors associated with the presence and level of infant PT antibodies at birth. Infant PT antibody at birth is the result of maternal placental transfer during gestation. An infant's level of PT antibody at birth is dependent on the maternal level. A recent study found only a quarter of infants at birth had PT antibody >5 EU[34]. Passively derived PT antibodies in infants can protect them from pertussis until they are able to be vaccinated themselves[35]. Factors previously shown to decrease infant PT antibody titers are lower maternal age, maternal HIV infection, and being born preterm[28,30,32,34,36]. However, few studies have been conducted in Asia and include multiple potential factors associated with infant PT antibody levels.

3.2 Identify maternal and household characteristics associated with maternal PT antibody titers.

This secondary aim was to estimate the association of maternal and household characteristics with maternal PT antibody titers at delivery. Maternal antibodies to PT in Nepal are either due to lingering antibodies from the childhood vaccination series or a pertussis infection. As there are no adolescent or adult boosters in Nepal high PT antibodies in mothers are likely due to recent in-

fection. High maternal antibodies can lead to protection from pertussis for both mother and infant[35]. However, many women do not have detectable PT antibody and the proportion with detectable antibody is thought to have decreased in recent decades[26]. A recent study found only a fifth of women at delivery with PT antibody levels > 5 EU/mL[34]. There are few data on the factors association with the presence or high levels of PT antibodies in pregnant and post-partum women. One study found no difference in PT antibody levels between HIV positive and HIV negative pregnant women. More data are needed in Asia on the prevalence of pertussis and factors that are associated with higher levels of PT antibody.

3.3 Identify infant, maternal, and household characteristics associated with PT antibody transfer ratio.

An additional secondary aim was to estimate the association of infant, maternal, and household factors on maternal to infant antibody transfer. Reduced PT antibody transport has most commonly been associated with lower gestational age[28,32,36]. While other factors such as maternal ethnicity have been found to affect maternal to infant IgG transport in general there are few data on specific factors associated with PT IgG transport efficiency, especially in Asia[36].

Section 3 – Laboratory Assays

Subsection 3.1 PCR

Real-time PCR testing was conducted at the University of Washington's Molecular Virology Laboratory according to previously published methods[37]. Two-target PCR was used to assess the presence of three Bordetella species: *B. pertussis, B. parapertussis, and B. brochispetica*. Multi-target PCR is more sensitive than single target and this particular assay may distinguish between *B. pertussis* and *B. parapertussis*. Positive controls were obtained using freeze-dried cultures from ATCC diluted 1:100 using Life Technologies' Hank's Balanced Salt Solution, extracted, and further diluted 1:1000 in TE pH7. Specimen and control DNA were extracted using MagNa Pure LC nucleic acid extraction method and stored at minus 80°C until testing.

The two independent pertussis sequence targets amplified were chromosomal repeated insertion sequence IS481 (IS) and the polymorphic pertussis toxin ptxA promoter region (PT) [Table 2.2]. There were 2 sets of primers for PT to accommodate small differences (2 bases) between pertussis strains (used in combination for one PCR reaction).

The pertussis PCR assay uses fluorescence resonance energy transfer SYBR green chemistry. SYBR green, a free-floating, DNA-binding dye, binds to double stranded DNA as it is replicated in the PCR reaction, the amount of fluorescence increases proportionally and can then be used to monitor the amplification of the target sequence. A commercial master mix, iQ SYBR green Supermix

(Bio-Rad, Hercules, CA), was used. The master mix (30 µl) was pipetted into each well of a 96-well plate. Each sample required 2 wells testing IS and PT primers with 10 µl of specimen added per well. The PCR cycle for PT amplification was as follows: 95°C for 3 minutes, followed by 45 cycles of 95°C for 10 seconds and 71°C for 45 seconds. The PCR cycle for IS amplification was as follows: 95°C for 2 minutes, followed by 45 cycles of 94°C for 30 seconds, 68°C for 30 seconds, and 72°C for 30 seconds and then 72°C for 5 minutes for extension.

TABLE 2.2 – PCR PRIMER TARGETS AND MELTING TEMPERATURES

PCR primer targets and Melting Temperatures					
Primer Type and Name	Sequence	Amplicon Length (bp)	*B. pertussis*	*B. parapertussis*	*B. bronchiseptica*
			Melting Temperatures		
IS*481* (IS)		182	85-86°C	-	-
IS-F	GATTCAATAGGTTGTATGCATGGTTC				
IS-R	TTCAGGCACACAAACTTGATGGGCG				
ptx promoter (PT)		189	87-88°C	89-89.5°C	90°C
PT-F1	CCAACGCGCATGCGTGCAGATTCG				
PT-F2	CCAACGCGTATGCGTGCGGATGCG				
PT-R1	CTCTGCGTTTTGATGGTGCCTATT				
PT-R2	CTCTGCGTTTCGGTGGTGCCTATT				

After completion of the 45th replication cycle, the melting points of the amplicons were measured in an iCycler (Bio-Rad) using a melt curve step that increases the temperature in small increments from approximately 60°C to 95°C, from which the melting temperatures of each of the Bordetella species can be measured. Each control should fall within the range of their individual expected temperatures to indicate a positive result.

A sample is interpreted as positive when the targets described in Table 2.2 have a melting temperature within the acceptable range and a Ct ≤42. A

sample is negative if none of the targets test positive or a single positive target is not reproducible.

Subsection 3.2 ELISA

An immunoglobulin G (IgG) anti-PT enzyme-linked immunosorbent assay (ELISA) was performed at Vanderbilt University School of Medicine according to previously described methods.[38]. The reference standard was in house pooled sera calibrated to pertussis antiserum (human), lot 3 (CBER3 [US Food and Drug Administration]).

First, PT antigen was coated on a Immulon 2HB microtiter plate. Following this was a 0.05 M carbonate-bicarbonate coating butter, which was incubated for 16-24 hours at 28°C[38]. Between each step the plate was washed 5 times in phosphate-buffered saline (PBS)-0.05% Tween 20. Next, eight 2-fold serial dilutions of serum were added to the plate and allowed to incubate for 2 hours at 28°C. Then an enzyme-conjugated goat anti-human IgG was added to the plate to detect PT-specific IgG antibodies; incubation occurred at 28°C for 16-24 hours. *Para*-nitrophenylphosphate (pNPP) substrate was added for a one hour incubation at 28°C. Lastly antibody concentrations were measured using a standard curve in SoftMax Pro/Molecular Devices software. The lower level of quantification (LOQ) was 1EU)/mL. The lower level of detection (LOD) was 2 EU/mL. However, titers below 10 EU/mL are non-reproducible and therefore not reported due to poor reliability.

Section 4 – Precision and Power Calculations

As the pertussis study was nested within the parent maternal influenza vaccination trial, sample size constraints are determined by the size of the larger trial. The sample size for the larger trial was based on the necessary power for each of the three primary outcomes and adjusting for multiple comparisons. The calculated necessary sample size was 1,850 mothers and their live born infants for an annual cohort.

Further, funding levels for the pertussis study limited the number of both nasal swab and blood specimens that could be funded. Lastly, due to delays in specimen shipping, not all results are available at present but will be available for final publication.

Subsection 4.1 – Aim 1 calculation

No sample size calculation was conducted *a priori* as this vaccine timing aim was not included in the original pertussis study. Given the coverage for at least 1 pertussis vaccine dose observed were able to calculate the percent vaccinated within +/- 2 percent, which is a precise estimate.

Subsection 4.2 - Aim 2 precision calculation

The sample size was fixed based on the aim of the parent trial to enroll approximately 1850 women and their infants in each cohort.

Limited population-based data on the incidence of pertussis in infants <6 months of age was available, especially in resource-poor settings. The best population-based estimates of disease came from the aP vaccine trials [Table 1.1]. The range of infant pertussis incidence estimates was 0.1 – 11.09 cases per 100 PY. Using these incidence estimates, our fixed sample size, and Equation 1, with type I error, α, set at 0.05, we calculated the range of relative precision available [Figure 2.4][39,40].

EQUATION 1

$$d = Z_{\left(1-\frac{\alpha}{2}\right)}\sqrt{\frac{P(1-P)}{n}} \qquad \text{[d = precision, p = incidence]}$$

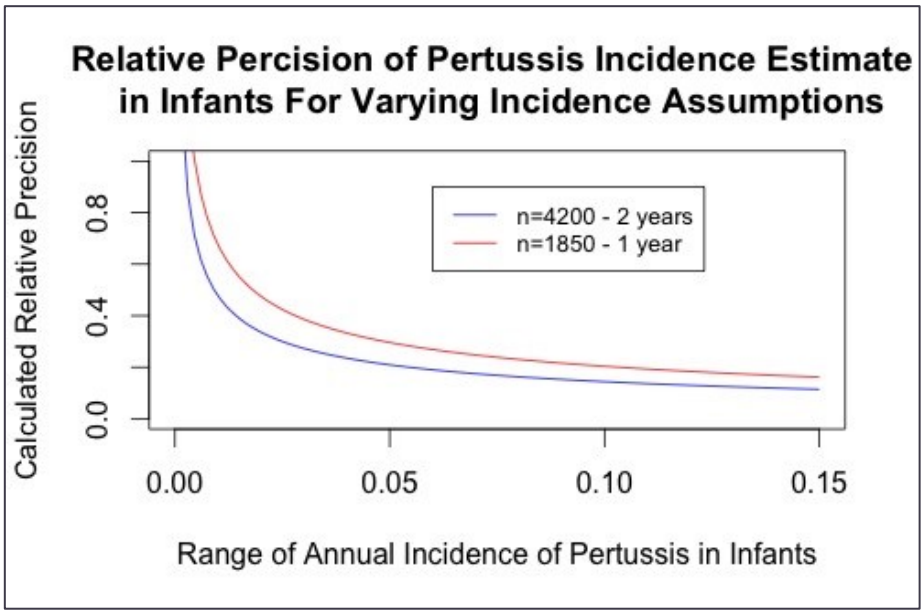

FIGURE 2.1 – PRECISION ESTIMATES FOR AIM 2

Two adjustments were made to the incidence estimates. First, as infants were only followed for 6 months we adjusted for infants contributing only half a year. Second, we expected 10% loss to follow-up and adjusted the sample size

needs accordingly. This loss to follow-up includes infant losses due to death and the time period following pertussis infection where the infant is no longer at risk. Figure 2.4 shows the relative precision estimates for various incidence scenarios. For example if the incidence was 0.04 cases per PY the relative precision of our incidence estimate would be 33.3%.

Subsection 4.3 - Aim 3 precision calculation

Aim 3 required a sample of mother infant pairs for whom sera were available near delivery. We expected to collect sera from 200 mother infant pairs. Using this sample size we calculated with what precision we would measure the ratio of paired infant-mother antibody titers. If we have an unknown variance of this ratio, we can calculate the precision, *d*, relative to the standard deviation using Equation 2. Given a sample size of 200 mother-infant pairs we would be able to estimate the ratio of infant to maternal antibody titers with a precision of 0.14 standard deviation (0.14σ) [Figure 2.5].

EQUATION 2

$$d = \frac{\sigma}{\sigma}\frac{Z_{\left(1-\frac{\alpha}{2}\right)}}{\sqrt{n}}$$ [d = precision, σ = standard deviation]

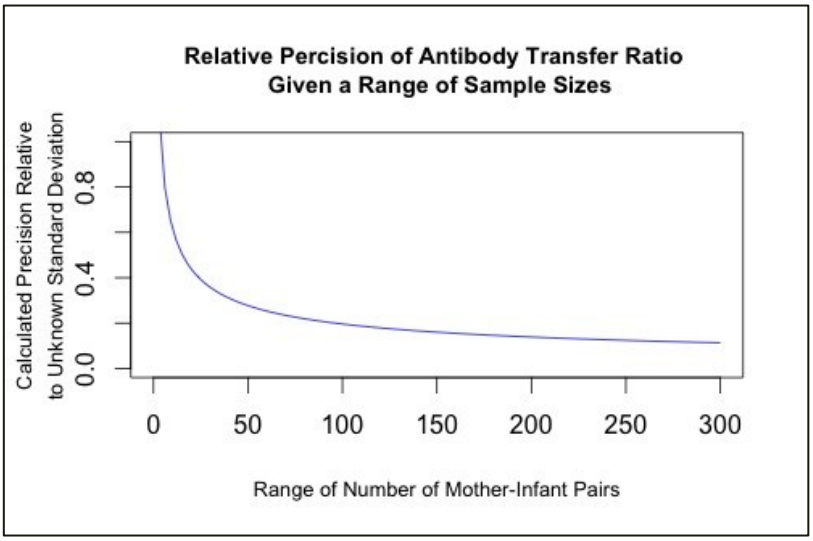

FIGURE 2.5 - PRECISION ESTIMATES FOR AIM 3

Section 5 – Statistical analyses

Statistical analyses were conducted in both Stata/SE 13.1 and R version 3.0.2 (2013-09-25).

Subsection 5.1 Aim 1

Vaccine coverage was calculated at 14 weeks (the recommended age for completion of the pertussis series) and 6 months (end of follow-up). For this analysis, infants were excluded if they were observed with weekly visits ending prior to 98 days (14 weeks) after birth to ensure all infants included had an opportunity to have recorded vaccinations at the recommended ages (6, 10, and 14 weeks). The primary outcomes were the proportion in each vaccination category (0, 1, 2, or 3 doses) at 14 weeks and 6 months. Equation 3 gives an example calculation for the number of infants fully vaccinated (3 DPT doses) at age 6 months.

EQUATION 3

$$DPT3_{6\ months} = \frac{\#\ infants\ with\ 3\ DPT\ doses\ at\ 6\ months}{\#\ infants\ followed\ minimum\ of\ 14\ weeks}$$

Survival analysis was used to measure the time to pertussis vaccination, separately for each of the 3 doses. Kaplan-Meier curves were constructed with a vaccination considered the event of interest. Infants were right-censored once they had the event of interest (specific vaccine dose) or had no further follow-up recorded (which included deaths as well as migrations).

Risk factors for time to 1st, 2nd, and 3rd pertussis vaccination were analyzed using a Cox-proportional hazards model. The recommended age of first pertussis vaccination dose, 42 days, was designated as time 0. Infants who were vaccinated prior to 42 days were assigned a date of vaccination immediately after time 0 ($1x10^{-6}$). The same adjustment made for lost-to follow-up was made for those infants with no follow-up after 42 days. Infants who had at least one follow-up visit but died before 42 days were excluded from the analysis. For the unadjusted model, hazard ratios, 95% confidence intervals (CI), and p-values from the Wald test of the maximum likelihood estimate were reported. Risk factors measuring similar characteristics were excluded to avoid any collinearity in the multivariate model. The multivariable model included adjusted hazard ratios, 95% confidence intervals (CI) and p-values from the Wald test of the maximum likelihood estimate. The proportionality assumption was tested through graphical diagnostics and testing based on scaled Schoenfeld residuals.

Risk factors for being vaccinated at 6 months for the 1st, 2nd, and 3rd pertussis doses were analyzed using a logistic regression model. Statistical significance was set at $p<0.05$ for all testing. All statistical analyses were conducted in R version 3.0.2 (2013-09-25).

Subsection 5.2 Aim 2

The primary outcome of Aim 2 was to estimate annual infant pertussis incidence in Nepal. An infant was considered at risk from birth until end of follow-up or until he contracted pertussis. Once an infant tested positive for pertussis he was not considered at risk for the remaining duration of follow-up. This analysis assumed that only 1 incident pertussis illness is possible in a 6-month period for infants. However, this assumption is consistent with current knowledge of no demonstrated pertussis re-infection within 6 months of natural infection. Therefore, while a participant may test positive over the course of several weeks, each subsequent positive pertussis test will be assumed related to the primary positive pertussis test.

The incidence was calculated as the number of *B. pertussis* cases per 1000 PY at risk [Equation 4]. 95% confidence intervals were constructed using Poisson exact confidence intervals. The same calculation was conducted to measure the incidence of *B. parapertussis*.

EQUATION 4

$$I_{pertussis} = \frac{\#\ incident\ pertussis\ cases}{1000\ person-years\ of\ follow-up}$$

Characteristics of all non-pertussis respiratory episodes were examined in comparison to pertussis episodes. T-tests were used to compare associations with continuous predictors and Fisher's exact test was used to compare categorical associations since the number of pertussis cases was small. Characteristics of pertussis cases were compared to non-pertussis cases. Continuous predictors were transformed to dichotomous factors to calculate incidence rate ratios for pertussis risk. The cutoff for statistical significance in all testing was $p<0.05$. All statistical analyses were conducted in Stata/SE 13.1.

Subsection 5.3 Aim 3

To quantify the level of maternal and infant PT antibody levels, geometric mean concentrations and bootstrapped-derived 95% confidence intervals were constructed separately for mothers and infants [Equation 5]. PT Antibody levels below the LOQ were assigned one-half of the assay LOQ (5 EU/mL).

EQUATION 5

$$GMC = \left[\frac{1}{n} * \sum_{i=1}^{n} \ln(x_i)\right] \qquad \text{[n = \# samples, x = antibody titer } (\frac{EU}{ml})\text{]}$$

Reverse cumulative distribution curves were created to visualize and compare the distribution of log transformed antibody titers for mothers and infants.[41] To examine differences in PT levels by infant, maternal, and household characteristics, non-parametric testing was performed for binary (Wilcoxon rank sum test with continuity correction) and nominal (Kruskal-Wallis rank sum test) variables. Non-parametric testing was performed because log transformation of the antibody titers did not result in a normal distribution. For this testing continu-

ous predictors were transformed to dichotomous and nominal factors. Bivariate and multivariate logistic regression models were used to assess the association of risk factors with the presence of PT antibodies separately for mothers and infants.

Placental transfer for each mother-infant pair was defined as the ratio of cord antibody concentration to maternal antibody concentration [Equation 6].

EQUATION 6

$$Transfer\ Ratio\ (TR)\ for\ 1\ Pair = \frac{C_{Cord}}{C_{Maternal}}$$

For primary Aim 3, the overall transfer ratio was the geometric mean of the infant to mother pair ratios [Equation 7]. 95% CI were constructed for the overall transfer ratio.

EQUATION 7

$$Overall\ TR = e^{\left[\frac{1}{n}*\sum_{i=1}^{n}\ln(TR_i)\right]} = \frac{GMC_{Cord}}{GMC_{Maternal}}$$

The ratio of infant to maternal PT GMC was also calculated in the subset of mother infant pairs where at least one of the pair had an antibody level above the LOQ. This subset therefore excluded pairs where both mother and infant had antibody level below the LOQ. Additional analysis of the transfer ratio for a secondary aim was restricted to the pairs in this subset.

Spearman's rank correlation rho was calculated for the correlation of mother and infant PT antibody. Unadjusted and adjusted linear regression mod-

els were created for examining the association of log ratio of infant to maternal PT antibody levels with infant, maternal, and household characteristics.

The cutoff for statistical significance in all testing was $p<0.05$. All statistical analyses were conducted in R version 3.0.2 (2013-09-25).

Subsection 5.4 Infant, Maternal, and Household Characteristics

At baseline, data on household structure was gathered, including age and sex of all household members. Households were categorized as crowded if 10 or more people resided in the home. The number of children under 5 years was transformed into a binary variable for households with 1 or fewer children <5 versus households with more than 1 child <5 years of age. Similarly, households were dichotomized into those with >3 children <15 years of age versus household with 3 or less children under 15 years. At enrollment women reported their literacy status (binary) and pregnancy history. The field workers identified their ethnicity (Pahadi or Madeshi) from names and observation. For parity analysis women were categorized as nulliparous or multiparous.

Twenty-five questions were asked to develop a construct to measure the socioeconomic status of households. The questions were the following: (1-3) construction materials for ground, first, and roof, (4) number of living and sleeping rooms, (5) water source, (6) type of latrine, (7) number of servants, (8-9) number of cattle and goats, (10-11) amount of *khet* and *bari* (measures of arable and non-arable land owned), (12-17) number of bullock carts, bicycles, motorcycles, cars/jeeps, trucks/buses, tractors, (18-23) number of clocks, radios, televi-

sions, satellite dishes, landline phones, mobile phones, (24) electricity in the home, and (25) household member working in another country. Responses for each of the 25 questions were dichotomized. Ground and first floor construction were counted as positive if construction materials were wood planks, brick or stone with mortar. Roof construction was coded as one if tin or cement were used. The presence of two or more living rooms was considered positive. For all other SES variables the presence of at least one (where applicable) item was considered as positive. The results were averaged (to account for differences in level of missing data in the denominator) and divided into SES quartiles for analysis.

Gestational age was measured using a woman's report of date of last menstrual period during pregnancy surveillance (an average of 3-4 weeks recall). Gestational ages <37 complete weeks were categorized as preterm. Birthweight was collected as soon as possible after birth using a digital scale [Tanita model BD-585, precision to nearest 10g]. Birthweights collected >72 hours after birth were excluded from the analysis of birthweight. Infants were categorized as low birthweight if weight was <2500 grams (g). Small for gestational age was calculated using the sex-specific 10^{th} percentile cut-off described by Alexander[42] and the 3rd percentile cut-off described by Oken.

Women were asked within how many hours of birth maternal breastfeeding was initiated (if any). Binary breastfeeding categories were created with women initiating breastfeeding within 1 hour compared to those initiating >1 hour post-delivery. Anthropometry measures were calculated from the 6-month weight

and length measurements. The z-scores for underweight (weight for age), stunting (length for age), and wasting (weight for length) were calculated using the WHO Child Growth Standards from the *igrowup* Stata package.

Section 6 – Quality Control

The principal investigators of the maternal influenza immunization trial have over 25 years of experience conducting community-based trials worldwide and in Nepal. This experience has led to the development of a tested quality control organizational system. Multiple levels of field staff were responsible for carrying out the study's data collection.

The first group, Flu Data Collectors (FDC), consisted of 81 married women who lived in the communities they covered. Each woman was responsible for 80-100 families in a given area. Prior to the trial start these FDCs underwent extensive training on the details of the trial protocol. FDC's specific responsibilities included identifying incident pregnancies through surveillance, working with participants' families to ensure they are notified as soon as possible after delivery, and conducting weekly morbidity home visits. During these visits they conduct a morbidity assessment and collected a nasal swab where indicated.

The Birth Assessment Team Members were responsible for collecting pregnancy outcomes as soon as possible after birth (with the aim of within 24 hours) and completing the 6 month post-partum follow-up.

Team Leader-Interviewers' (TLI) duties included enrolling women, obtaining informed consent, pregnancy follow-up and post-delivery interviews. In addition they retrieved nasal swab specimens weekly from the FDCs and supervised FDCs through weekly meetings.

Field Supervisors were responsible for the entire study area and were the bridge between the field headquarters and the staff in the field. Field Supervisors were integral to the project's training program and provided overall management support to the project. Field Supervisors oversaw TLIs through weekly meetings and random checks of TLI reports. They were also responsible for conducting verbal autopsies.

Auxiliary nurse midwives (ANMs) administered the study vaccine, monitored the cold chain during field transport, and collected, processed, and shipped biospecimens.

The Field Coordinator and Field Manager oversaw the entire field operation. They supervised the FSs and helped to resolve issues as they arose. The Field Coordinator and Field Manager helped to develop the data collection instruments including pre-testing them in the field. They were also responsible for interviewing, hiring, and training all levels of field workers working below them. The Field Manager was specifically responsible for managing the vaccine supply including transport to and from Kathmandu.

The Medical Director, based in Kathmandu, was responsible for all field activities and was a liaison between U.S. based investigators. The Medical Direc-

tor reviewed all adverse event report forms and led study form translations (Nepali – English).

Section 7 – Data Management

The parent maternal influenza vaccination study personnel had responsibility for the entirety of field data collection and management. The field site had extensive experience in managing data from large-scale field trials. Project Headquarters office was in Kathmandu and Field Headquarters were in Sarlahi. Data collection forms were developed by investigators in the US and Nepal and underwent extensive field testing, including focus groups before forms were finalized. English versions of the data collection and consent forms were translated to Nepali and then back to English to ensure meaning of terms was not lost in translation.

Subsection 7.1 Form Generation

Each participant was assigned a unique designated identification number used on all patient-specific study forms and specimens. After the baseline census, all women eligible for pregnancy surveillance were entered into the database used to track women who were monitored for incident pregnancies. Once pregnant women were enrolled in the study (and vaccinated), individual forms were generated for her data collection needs through 6 months post-partum.

All data forms completed at field sites were transported to the Field Headquarters and then sent to Project Headquarters on a regular basis.

Subsection 7.2 Data Entry

A Data Center manager, responsible for supervision of Data Entry Operators and generating regular data reports, oversaw the data center. Data Operators entered data on screens that emulated the paper data collection forms at Project Headquarters. The Data Operators were responsible for data entry, editing, and form storage management. The computerized forms were custom-programmed with numerous levels of data validation to identify missing, inconsistent, or out of range data. In addition all form header information was double entered and select form data were double entered. Entering forms was required in a fixed order to ensure that forms in a data collection sequence were not missing.

Once a form was entered, it was stored in a household specific folder that ultimately contained all data on that participant and her infant. The folder cover listed the name, NNIPS number, and address of the participant. Once a form was entered the Data Entry Operator stamped the date on the form and also this information was stored in the electronic database. Folders were filed by NNIPS number in a secure area.

Subsection 7.3 Identification and Resolution of Data Inconsistencies

At the time of data entry, computerized checks were conducted to identify missing, inconsistent or out of range data. A data error query was produced and either resolved within the data center by reviewing other data collected on the

individual, or the query was returned to the field with a copy of the original form for resolution, either at the clinic or by visiting the participant to ascertain the appropriate data to resolve the problem. A specific error correction form was only used for error corrections that originated in the field or were sent back to the field from the data center. Some errors were resolved in the data center without using the form. If it was determined that corrections were needed on the source data form itself (date error for example), the field staff put a single line through the error, initialed and dated the change. An electronic audit trail tracked any change made to the database after initial data entry.

An external monitoring group visited the site once every two months to monitor the study's compliance with Good Clinical Practice (GCP). Part of this process involved random checking and verification of form logic and comparison of paper form data to electronic data.

Subsection 7.4 Electronic Data Back-Up and Transfer

Study data were backed up daily. A master copy of the data was backed up on a weekly basis and kept in an alternate location in case of unexpected damage (e.g. fire) at the main data site. The data were transmitted periodically (compressed and encrypted) to Baltimore from Nepal.

Subsection 7.5 Data Security and Protection of Subject Confidentiality

All information from study subjects was kept confidential. Only those au-

thorized had access to these data. All forms were held a secured set of rooms at study headquarters in Kathmandu. Access to identifiable records was limited to study staff and their grounds for employment was contingent on their maintaining the security of study records and any identifiable information. Computer files were secured via logon password protection for study accounts and database files. The Data Center was located in a secured location in the Project Headquarters in Kathmandu, locked and/or guarded at all times. All data files transferred to Baltimore via encrypted software were kept on our Johns Hopkins server behind the local firewall.

The two academic centers who processed the blood and nasal swab specimens received specimens labeled with a specimen ID number and no identifiable information. Once specimens were processed, the data, by specimen ID, was entered into an Excel spreadsheet. The Excel document was transmitted to Baltimore where the specimen IDs were linked to participant IDs for analysis.

Identifiers will be kept on all data files until the study is closed out. Primary data collection sources will be kept for at least 3 years following the publication of the primary results from this trial. Once that time elapses and the electronic data files are fully cleaned, paper forms will be destroyed.

Section 8 – Human Subjects

The study was nested within the maternal influenza trial, which included testing specimens for other pathogens beyond influenza. An amendment was approved for the maternal influenza trial research plan to include pertussis testing

of nasal swab and blood specimens. The study was approved by the following Institutional Review Boards (IRBs):

1. Cincinnati Children's Medical Center (FWA #00002988)
2. Johns Hopkins Bloomberg School of Public Health (JHSPH) (FWA #00000287)
3. Seattle Children's Hospital (FWA #00002443)
4. Institute of Medicine at Tribhuvan University/Nepal Health Research Council (FWA #00000957)

Subsection 8.1 Confidentiality

Data collection forms will be maintained in locked cabinets in the guarded Kathmandu study headquarters. Electronic databases will be stored only at the Kathmandu site and in Baltimore. Access will be granted only to those with valid login and passwords. Study identification numbers will be used whenever possible in lieu of a personal identifier. Identification will be kept on biospecimens until testing is complete but include only ID numbers, not names. Data collection sources will be kept for at least 3 years following the publication of the primary results from this trial. Once that time elapses and the electronic data files are fully cleaned, paper forms will be destroyed.

Subsection 8.2 Consent

Consent to participate in the study occurred at multiple stages. Prior to the start of the study senior investigators met with community leaders and health care providers to discuss the proposed trial and answer specific questions. During the baseline census, at all households where a married woman 15-40 years of age resided, the head of household was read an informed consent script. Women residing in consenting households were read an individual informed consent script, which included consent to participate in pregnancy surveillance and to participate in the trial if a pregnancy occurred. If a woman did not consent the household was not contacted further. Household and individual consent was documented on the household enrollment roster. Once a woman delivered her child she was asked if she agreed to enroll her infant although not using the full consent script. A woman's consent for herself and/or her child was obtained again at the time of potential nasal swab and blood collection. The consent was documented on the participant-specific specimen collection form.

Verbal consent was the method used for all participants and a waiver of written consent was approved by all participating IRB's. One reason for non-written consent is that the majority of the participants were illiterate. Moreover, signatures or fingerprints in Nepal are generally reserved for important financial transactions and may cause great suspicion regarding the intentions of the study team. Once the verbal consent was read to a participant and the participant verbally consented, the field worker recorded on the form that the consent was read,

any questions were answered, and the participant's agreement or refusal to participate.

Subsection 8.3 Risks

Risks to participants were similar to those for a comprehensive physical exam. The risks included potential privacy loss, slight discomfort during nasal swab and blood specimen collection, and discomfort and adverse reactions from the influenza vaccine.

Many steps were taken to minimize these risks. Interviews were conducted in a private area of the home and data were stored in a secure environment to protect the risk of privacy invasion. The risk of discomfort during various specimen collection was minimized by using well-tested and sterile techniques and monitoring participants for adverse effects. Discomfort during vaccine administration was minimized by using well-trained vaccinators skilled in sterile technique and close monitoring for adverse events following immunization.

Subsection 8.4 Benefits

All participants in the study received potential benefits. Women randomized to receive the influenza vaccine may have protected themselves and their infants from influenza illness. Those women randomized to receive placebo received an influenza vaccine at the completion of follow-up.

Women also received improved antenatal services compared to the local standard of care including 90 days of iron-folic acid supplements (if not yet re-

ceived from local health facility), single dose 400 mg albendazole (deworming) (if not yet received from local health facility), tetanus toxoid vaccination if indicated, tube of 4% chlorhexidine ointment for the umbilical cord, and educational messages on nutrition and safe birthing practices.

There were no extra direct benefits to those who participated in the study. Society will benefit from the study through understanding of the effect of maternal influenza immunization on mothers and infants. Further, society will benefit from the pertussis study through understanding of the burden and risk factors of pertussis in infants. The knowledge gained may help influence maternal and child immunization policies and programs in Nepal and throughout the world.

Subsection 8.5 Research Burden

Households incurred a small burden through participation in this study. Households where women of reproductive age resided were visited at baseline and then every 5 weeks to monitor for new pregnancies. If a woman became pregnant and enrolled in the study the household was visited weekly through 6 months post-partum. Based on past experience conducting studies in this population, weekly visits are not considered a significant burden, as households are rarely hesitant to participate in these visits.

Subsection 8.6 Payments

No payment were made to study participants.

Subsection 8.7 Monitoring

The maternal influenza trial had two committees to provide organizational support. The Executive Committee was responsible for the scientific and administrative conduct of the trial. The Field Operations Committee was responsible for implementing the study protocol under the direction of the Executive Committee.

The study Data Safety and Monitoring Board (DSMB) provided external oversight of the safety of the intervention and was responsible for meeting yearly to review the data. The DSMB conclusions were written and provided to the Executive Committee. The lead investigator then forwarded the DSMB conclusions to all participating IRBs. The DSMB included members with the following expertise: biostatistics, obstetrics, pediatrics, epidemiology, and research ethics. Senior members of the investigator team were non-voting members of the DSMB.

An external clinical research organization also monitored the trial with a review of data, procedures, and IRB documents every two months for the duration of the trial.

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CHAPTER THREE

Pertussis Vaccination Timing and Factors Associated with Delay in Sarlahi, Nepal: A Population-Based Prospective Cohort

Authors

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Abstract

Background: Pertussis is a significant contributor to infant morbidity and mortality in low and middle-income countries where the majority of cases occur. Pertussis vaccination in infancy is the sole prevention strategy in many countries supported by the WHO's Expanded Programme on Immunization. Data capturing the timing of and delays in vaccination in first 6 months of life, when severe disease occurs, are lacking.

Objective: To estimate the time to pertussis vaccination and risk factors for delay in infants <6 months of age in Sarlahi District, Nepal.

Design/Methods: Infants, enrolled in a randomized controlled trial of maternal influenza vaccination during pregnancy, were visited in their homes weekly to ascertain if any vaccinations had been given in the prior week from birth through age 6 months. Infant, maternal, and household characteristics were captured at enrollment and birth. DPT1, DPT2, and DPT3 vaccination coverage at 14 weeks and 6 months was estimated; a logistic regression model was used to examine risk factors for delay. Time to vaccination was estimated through Kaplan-Meier curves; a cox-proportional hazards model was used for risk factor assessment.

Results: The median age of DPT1 receipt was 18 weeks, which translates to a 12-week delay from the recommended schedule. Only 7% of infants had received DPT3 by age 6 months. Infants born small-for-gestational age or whose mothers were of Madeshi ethnicity or failed to initiate early breastfeeding were at high risk for delayed immunization.

Conclusion: A substantial delay in receipt of the primary pertussis vaccination series was found in a prospective population-based cohort in Sarlahi District, Nepal. In addition to targeting groups most at risk for immunization delay, countries should consider improved immunization monitoring to capture these delays in infants <6 months.

Introduction

Immunization is the primary means of pertussis prevention, dramatically reducing pertussis burden since pertussis vaccine's initial introduction in the 1940s[1]. The World Health Organization (WHO) estimates that since the end of the 1980s, 80% of children worldwide have received pertussis vaccines, preventing approximately 38 million cases and 600,000 deaths annually[2]. Worldwide childhood pertussis vaccination coverage rates have increased substantially since the introduction of the Expanded Programme on Immunization (EPI) in 1974[3]. The WHO recommends a three dose primary series, in combination with diphtheria and tetanus, at 6 weeks (DPT1), 10-14 weeks (DPT2), and 14-18 weeks (DPT3). Prior to EPI global DPT3 coverage was less than 5%; DPT3 coverage in 2012 was 83% (measured at 12-23 months of age)[4,5]. Despite tremendous progress, global coverage remains below the target of 90% DPT3 coverage[6].

In recent years several high-income countries have experienced a resurgence of pertussis, particularly in infants and adolescents[7,8]. Several factors are thought to contribute to the epidemic levels including genetic changes in the *Bordetella pertussis* bacteria, waning immunity, lower and shorter duration of immunity from acellular versus whole cell pertussis vaccines, heightened awareness, increased surveillance, and vaccination delay and refusal[9-18].

Delay in vaccination in the primary series is especially important for infants who are at greatest risk for severe morbidity and mortality[8,19]. While infants might

have partial protection from passive transfer of pertussis antibodies from their mothers, this immunity eventually wanes, requiring active immunization.

The distribution of pertussis burden is not equal with 95% of cases thought to occur in low-income countries resulting in 4% of <5 deaths in Southeast Asia attributable to pertussis[6,20]. In Nepal DPT3 vaccine coverage increased from 54% of children fully vaccinated by 12-23 months of age in 1995 to 90% in 2012[21]. Even though this coverage is relatively high this measure does not capture vaccine delays in the first 6 months of life when infants are at high risk. Recent studies found DPT3 coverage at 6 months in low and middle-income countries was just 36%[22]. Population-based data of early vaccination coverage using active surveillance in low-income countries are lacking. This prospective, population-based cohort study aimed to estimate pertussis vaccination timing and risk factors for delay in the first 6 months of life in Sarlahi, Nepal. This information is important for policy makers to understand potential delays in vaccination and which populations are most at risk for targeted interventions to improve timeliness of uptake.

Methods

Settings and population

The setting of the study was Sarlahi District, located in the central terai (low lying plains) region of Nepal. The study was nested within a randomized controlled trial of maternal influenza vaccination during pregnancy. At the start of the trial, prevalent pregnancies were identified through a survey census of all households in the catchment area. For the duration of the trial field workers visited all households in the community where married women (15 – 40 years) resided every 5 weeks for surveillance of incident pregnancies. Once a pregnancy was identified women were asked for their consent to participate in the trial. Over a two-year period between April 25, 2011 and April 24, 2013 women between 17-34 weeks gestation were randomized and vaccinated with either an influenza vaccine or placebo. All participants received ancillary benefits, which included a 90-day supply of iron-folic acid tablets, deworming medication (single dose of albendazole), clean birthing kit, chlorhexidine ointment for umbilical cord care, a tetanus toxoid vaccine, if indicated, and health education messages, in addition to antenatal services according to the local standard of care. The study was a population-based prospective cohort of infants followed from birth through 6 months post-partum. Ethical approval for the study was obtained from the Johns Hopkins Bloomberg School of Public Health Institutional Review Board and the local ethical review board (Institute of Medicine at Tribhuvan University/Nepal Health Research Council).

Data collection

At baseline information was collected on household structure, socioeconomic status, and demographics. At study enrollment, date of last menstrual period and pregnancy history data were collected. As soon as possible after delivery the mother and infant were visited to collect detailed birth information including infant weight and breastfeeding status. From birth through 6 months postpartum (180 days) infants were visited weekly by a field worker who recorded any vaccinations and which specific ones were received in the past 7 days.

Analytic Dataset

Infants were included in this analysis if they were followed for any length (0 to 180 days) during a 2 year-period from May 24, 2011 to January 14, 2014. [Complete data from trial pending]. Of 3,689 women vaccinated, 12 were missing a maternal delivery assessment (due to incomplete data entry at time of analysis) leaving 3,677 women with delivery assessment information [Appendix 3.1]. There were 5 maternal deaths, 9 miscarriages, and 4 abortions. Twenty-five twin pregnancies were excluded from the analysis. Of the 3,634 singleton births, there were 3,571 live births, 58 stillbirths, and 5 had missing birth information. Of the 3,571 live singleton births, 23 died with no weekly morbidity follow-up in the neonatal period, 145 were live born but had no follow-up and 4 were followed up only after 180 days and thus were excluded from the analysis. The final dataset consists of 3,399 infants with at least one follow-up visit during the first 6 months.

At baseline, data on household structure was gathered, including age and sex of all household members. Households were categorized as crowded if 10 or more people resided in the home. The number of children under 5 years was

transformed into a binary variable for households with 1 or less children <5 versus households with >1 child <5. Similarly, households were dichotomized into those with >3 children <15 versus households with 3 or less children under 15 years. At enrollment women reported their literacy status (binary) and pregnancy history. The field workers identified their ethnicity (Pahadi or Madeshi) from names and observation. For parity analysis women were categorized as nulliparous or multiparous. Twenty-five questions were asked to develop a construct to measure the socioeconomic status (SES) of households. The questions were the following: (1-3) construction materials for ground, first, and roof, (4) number of living and sleeping rooms, (5) water source, (6) type of latrine, (7) number of servants, (8-9) number of cattle and goats, (10-11) amount of *khet* and *bari* (measures of arable and non-arable land owned), (12-17) number of bullock carts, bicycles, motorcycles, cars/jeeps, trucks/buses, tractors, (18-23) number of clocks, radios, televisions, satellite dishes, landline phones, mobile phones, (24) electricity in home, and (25) household member working in another country. Responses for each of the 25 questions were dichotomized. Ground and first floor construction were counted as positive if construction materials were wood planks, brick or stone with mortar. Roof construction was coded as one if tin or cement were used. The presence of two or more living rooms was considered positive. For all other SES variables the presence of at least one (where applicable) item was considered as positive. The SES variable was the percent of items on the 25-item scale that were positive. If any items were missing, the score was

the percent positive out of the number of non-missing items. These percentages were divided into SES quartiles for analysis.

Gestational age was measured using a woman's report of date of last menstrual period during pregnancy surveillance (an average of 3-4 weeks recall). Gestational ages <37 complete weeks were categorized as preterm. Birthweight was collected as soon as possible after birth using a digital scale [Tanita model BD-585, precision to nearest 10g]. Birthweights collected >72 hours after birth were excluded from the analysis of birthweight. Infants were categorized as low birthweight if weight was <2500 grams (g). Small for gestational age (SGA) was calculated using the sex-specific 10th percentile cut-off described by Alexander[23]. Women were asked within how many hours of birth maternal breastfeeding was initiated (if any). Binary breastfeeding categories were created with women initiating breastfeeding within 1 hour compared to those initiating >1 hour post-delivery.

Statistical Analysis

Vaccine coverage was calculated at 14 weeks (the recommended age for completion of the pertussis series) and 6 months (end of follow-up). For this analysis, infants were excluded if they were observed with weekly visits ending prior to 98 days (14 weeks) after birth to ensure all infants included had an opportunity to have recorded vaccinations at the recommended vaccination ages (6, 10, and 14 weeks). The primary outcome was the proportion in each vaccination category at 14 weeks and 6 months.

Survival analysis was used to measure the time to pertussis vaccination, separately for each of the 3 doses. Kaplan-Meier curves were constructed with a vaccination considered the event of interest. Infants were right-censored once they had the event of interest (specific vaccine dose) or had no further follow-up recorded (which included deaths as well as migrations).

Infant, maternal, and household risk factors for time to 1st, 2nd, and 3rd pertussis vaccination were analyzed using a Cox-proportional hazards model. The recommended age of first pertussis vaccination dose, 42 days, was designated as time 0. Infants who were vaccinated prior to 42 days were assigned a date of vaccination immediately after time 0 ($1x10^{-6}$). The same adjustment made for loss-to follow-up was made for those infants with no follow-up after 42 days. Infants who had at least one follow-up visit but died before 42 days were excluded from the analysis. For the unadjusted model, hazard ratios, 95% confidence intervals (CI), and p-values from the Wald test of the maximum likelihood estimate were reported. Risk factors measuring similar characteristics were excluded to avoid any collinearity in the multivariate model. The multivariable model included adjusted hazard ratios, 95% CI and p-values from the Wald test of the maximum likelihood estimate. The proportionality assumption was tested through graphical diagnostics and testing based on scaled Schoenfeld residuals.

Risk factors for being vaccinated at 6 months for the 1st, 2nd, and 3rd pertussis doses were analyzed using a logistic regression model including infant, maternal and household risk factors.

Statistical significance was set at $p<0.05$ for all testing. All statistical analyses were conducted in R version 3.0.2 (2013-09-25).

Results

3,339 infants were visited at least once from age 0-180 days. The visit dates ranged from May 24, 2011 to January 14, 2014. For binary pertussis coverage and logistic regression estimates, 266 infants were excluded due to having no data at or beyond 98 days (age when 3rd dose recommended). 3,133 infants were observed to at least age 98 days. For these infants, the mean age of last follow-up visit was 168 days (SD = 16.1). Mean ages of first, second, and third pertussis doses were 83, 114, and 128 days, respectively among those who had observations up to 98 days or beyond. A subset of infants was vaccinated prior to the recommended age of vaccination. 106 infants were vaccinated with a pertussis vaccine prior to age 42 days. For the 2nd and third dose the numbers of early vaccinated were 39 and 20, respectively.

The majority (60%) of infants had received no pertussis immunization by age 14 weeks, the recommended age for completion of all 3 doses. By age 6 months, 43% of infants remained unvaccinated for pertussis [Figure 3.1]. Only 1% were fully vaccinated by 14 weeks with the percentage increasing slightly to 7% fully vaccinated by 6 months.

The median age at first pertussis vaccination, estimated using survival curves, was 128 days (95% CI: 124 -134) [Figure 3.2].

Cox proportional hazard models were used to estimate the relative hazard of being unvaccinated in unadjusted (bivariable) and adjusted (multivariable) models [Table 3.1]. The proportionality assumption was tested through graphical

diagnostics [Appendix 3.2] and testing based on scaled Schoenfeld residuals [Appendix 3.3]. Parity, SES, and the two childhood crowding-associated variables all had statistical significance for non-proportionality. Modeling a time interaction term for these variables showed no significant interaction so variation with time was not included in the model.

Factors associated with a higher hazard of being unvaccinated in bivariable models were being born SGA, failure to initiate breastfeeding in first hour after birth, maternal illiteracy and Madeshi ethnicity, and household crowding both for all household members and members under the age of 15 years. The strongest associations were for ethnicity (HR 1.35; 95% CI: 1.23 - 1.50) and breastfeeding initiation (HR 1.18; 95% CI: 1.07 - 1.29). A multivariable model was constructed removing variables measuring similar outcomes as those included in the final model (birthweight, crowding, children <5 years). Ethnicity, breastfeeding, and SGA remained significant in the multivariable model.

For the model of time to second vaccination, SGA, breastfeeding, ethnicity, and crowding remained statistically significant in the bivariable model [Appendix 3.4]. In the multivariable model only SGA remained statistically significant. Modeling time to third vaccination showed only SGA and breastfeeding were statistically significant in the bivariable model [Appendix 3.5]. In the full model both of these factors remained significant and children <15 rose to statistical significance. Of note however is that the models to second and third vaccination had less power to detect statistical significance given lower numbers for these outcomes compared to the first vaccination.

Logistic regression was also used to look at the binary outcome of having at least 1, 2, or 3 doses completed by age 6 months. In the bivariable logistic model for having received at least 1 pertussis dose the same factors were significant as in the Cox proportional hazards model; the exception was literacy, which was not statistically significant [Appendix 3.6]. Only ethnicity remained statistically significant in the full model. SGA, ethnicity, and crowding were significant in the model estimating the odds of at least 2 doses completed by age 6 months. Only SGA status was a statistically significant predictor of having at least 2 doses by 6 months in the adjusted model [Appendix 3.7]. In the unadjusted model for fully vaccinated (all 3 doses) at 6 months, SGA, breastfeeding, and SES were statistically significant [Appendix 3.8]. In the full model only early breastfeeding initiation was a statistically significant predictor of full vaccination status at 6 months.

Discussion

Substantial delays in uptake of the primary pertussis vaccination series were found in infants <6 months in Sarlahi District, Nepal. The median age of DPT1 vaccination was 18 weeks, a 12-week delay from the recommended age of 6 weeks. Only 7% of infants were fully vaccinated with DPT3 by age 6 months. This significant delay is not captured by WHO estimates. The most recent Nepal data from 2013 give DPT1 and DPT3 coverage at 94% and 92%, respectively[4]. In the central terai region, where Sarlahi District is located, 96%, 92%, and 87% of children have received one, two, and three doses, respectively, of DPT by ages 12-23 months[24]. While these official coverage estimates are high, our data show many infants receive vaccines on a delayed schedule. Globally, WHO and UNICEF use officially reported data and sample survey data to measure DPT coverage of children 12-23 months[3]. As a result, if there are substantial delays in vaccination, but DPT vaccines are complete by age 2, a child is still considered as vaccinated on schedule. In the U.S. and elsewhere standard national reporting statistics obscure delays when infants are at risk for vaccine-preventable diseases[22,25-27]. National-level reporting may also mask within-country variation in vaccination timeliness[22].

Our finding of significant vaccination delay is consistent with data from other countries. For example, Japan's reported DPT3 coverage was 98% in 2013, however data from a representative city in Japan showed less than 50% DPT coverage by age 12 months[26]. In the U.S. a study found almost half of children had some delay in receiving a DTaP vaccine dose and 16% were delayed in

vaccine receipt for more than 6 months in the first two years[25] despite national DPT3 coverage at 94% in 2013. A longitudinal study in Ghana reported that while DPT3 coverage was 95% at 12 months, only 10% of infants were vaccinated within 1 week of the scheduled time (14 weeks); the median delay for DPT3 was 4 weeks [28]. A study examining the timing of vaccination in low and middle income countries, based on surveys and imputed data, found median DPT1 coverage at 6 months was 82% (95% CI: 67-89%) and DPT3 was 36% (95% CI: 23-54)[22]. Our data from Nepal of DPT1 (57%) and DPT3 (7%) coverage at 6 months is significantly lower than these estimates from other similar countries. An interpretation of this is that Nepal may in reality have increased vaccination delay compared to similar countries. An alternative interpretation is that in general survey and imputed data lead to an overestimation of coverage. Our weekly active surveillance data might have led to a more precise and unbiased estimation of vaccination coverage.

Despite the vaccination delays found, Nepal considers its National Immunization Program a high priority with the country on track to achieve Millennium Development Goal 4 on child mortality reduction[29,30]. Timeliness of childhood vaccination is crucial in Nepal since no booster immunizations are recommended for adolescents and adults, including pregnant women. The delay is important as it leaves infants at risk for pertussis. Children who are unimmunized or under immunized are at increased risk for pertussis and pertussis hospitalization compared to their more fully immunized peers[16,26,31-33].

We modeled vaccination delay as both time to vaccination and vaccination status at 6 months. More factors reached statistical significance in the models of time to first pertussis vaccination or having at least 1 vaccine. This is likely due to the increased power of these models with more outcomes of interest observed in comparison to models for DPT2 or DPT3. In bivariate logistic (status at 6 months) and cox-proportional hazard (time to vaccination) models, being born SGA, failure to initiate breastfeeding in the first hour of birth, Madeshi ethnicity, and household crowding were all associated with vaccination delay. Maternal illiteracy was statistically significant in the cox-proportional model alone. When examining the risk factors in combination, SGA status, breastfeeding status, and ethnicity remained the most important predictors of vaccination delay. We found no difference in vaccination status by sex or birth order in contrast to that found in the Nepal Demographic and health survey[24]. One reason why these factors might contribute to vaccination delay is that they are markers for poorer access to health services. Mothers who have lower utilization of antenatal care might be at higher risk for infants born SGA and have had less exposure to the importance of early initiation of breastfeeding. Women of Madeshi ethnicity have less mobility and empowerment, and are therefore less likely to access health care resources for themselves and their children. These same factors might also lead to lower access of infant check-up visits where infants have an opportunity for vaccination.

Reasons for vaccination delay in low and middle-income countries include poor immunization supply, lack of access to health services, and family charac-

teristics[27,28,34]. Parents may also be hesitant to vaccinate or not view the costs involved with vaccination worth the benefit. In Ghana infants who were poorer, had less educated mothers, and lived in rural versus urban areas were significantly more likely to delay vaccination compared to urban infants whose mothers were educated and in a higher income groups[28]. A study of 31 low and middle income countries also found that children in poorer families and families with more than one child were at increased risk for vaccination delay[27]. In the U.S. vaccination delay is associated with a mother who is unmarried, less educated, non-Hispanic black, and uses public vaccination providers[25]. In comparison in our Nepal population, literacy, a surrogate for maternal education was only significant in the bivariable cox-proportional hazard model of time to first vaccination. Low socioeconomic status was a significant predictor in being non-fully vaccinated at 6 months only in an unadjusted model. Parity was not a significant predictor in our population, which differs from previous findings. Our study provides an improved understanding of Nepal-specific factors contributing to vaccination delay that can help programs focus on at-risk populations to increase on-time vaccination.

A limitation of our study is that our surveillance extended only for the first 6 months of life. We were not able to capture the timing of vaccination receipt to age 12 months. We cannot provide if or when vaccines were received to capture the full delay. This limited the direct comparability of our data to official data reported at age 1 year. The infants in our surveillance population might have reached the officially reported coverage by 12 months. Official reporting in some

countries may overestimate the coverage in part to reach donor targets such as GAVI's immunization services support (ISS)[3]. While, GAVI is the largest contributor to Nepal's immunization budget providing 46% of necessary funds, it is not possible to know why our 6 month coverage data do not correspond to those at 12-23 months[35]. The most likely explanation is that there is catch-up of vaccination beyond 6 months of age. Another may be that rural Sarlahi may not represent other parts of the country, especially urban areas, in terms of coverage data.

Another limitation of our study was that recording of vaccine receipt was reported by parents and not confirmed by review of immunization cards. This could have led to misclassification if the parent reported an incorrect vaccine. Overestimation of coverage could have occurred if parents over reported vaccine receipt or underestimation if parents forgot or were unaware of a vaccine the infant previously received. However, parents were visited in their homes on a weekly basis limiting the chance for recall bias although it is possible that they did not correctly recall the type of vaccine provided.

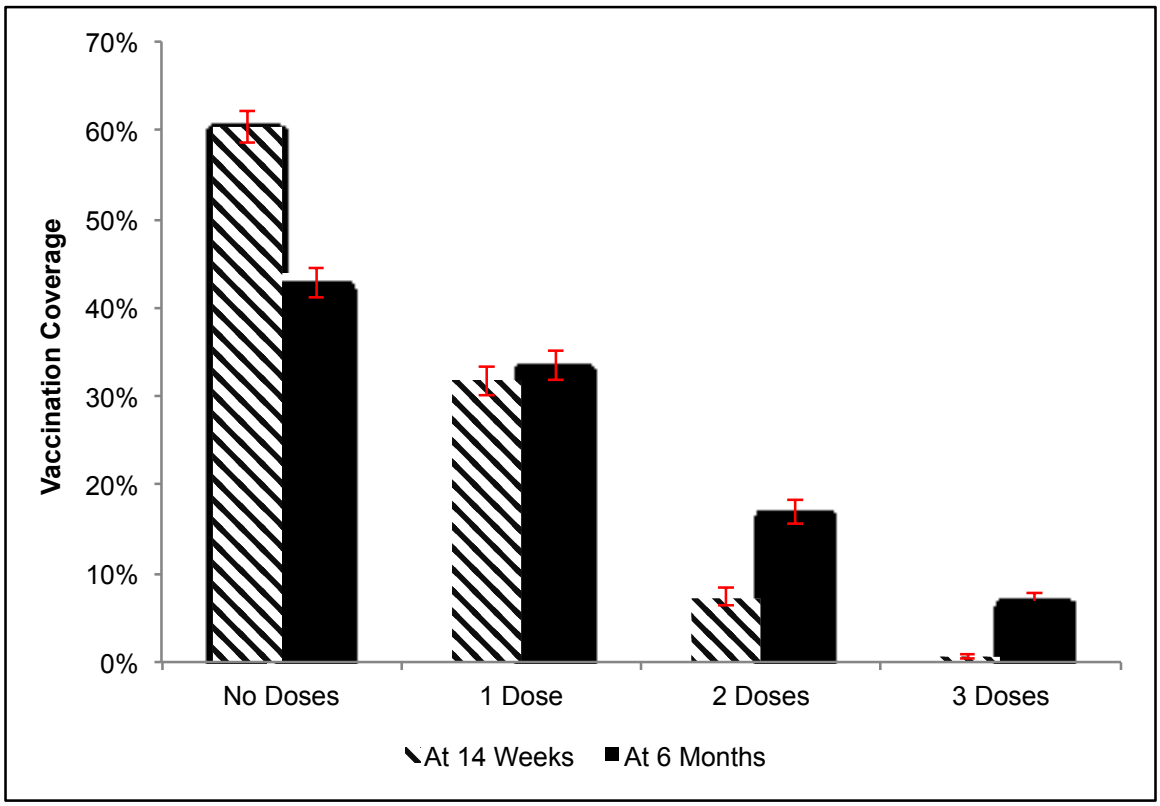
We have no qualitative data from parents on their perspective of the vaccination status of their children. While we were able to quantitatively examine risk factors we are limited in understanding the underlying reasons for vaccination delay.

Strengths of this study are that it was a population-based cohort study following infants prospectively from birth through age 6 months. Surveillance was conducted at high frequency (weekly) in the homes of all participants. The capture of time of vaccination provides important information on vaccine delay for

Nepal policy makers. While the population was limited to one district in Nepal the results are likely generalizable to most of the Nepalese population. The majority of the Nepali population lives in the terai region, where Sarlahi District is located. Infant health and vaccination indictors are similar to country-wide estimates[24]. Sarlahi District, which borders India near sea level, has similar living conditions to many populations of South Asia[36].

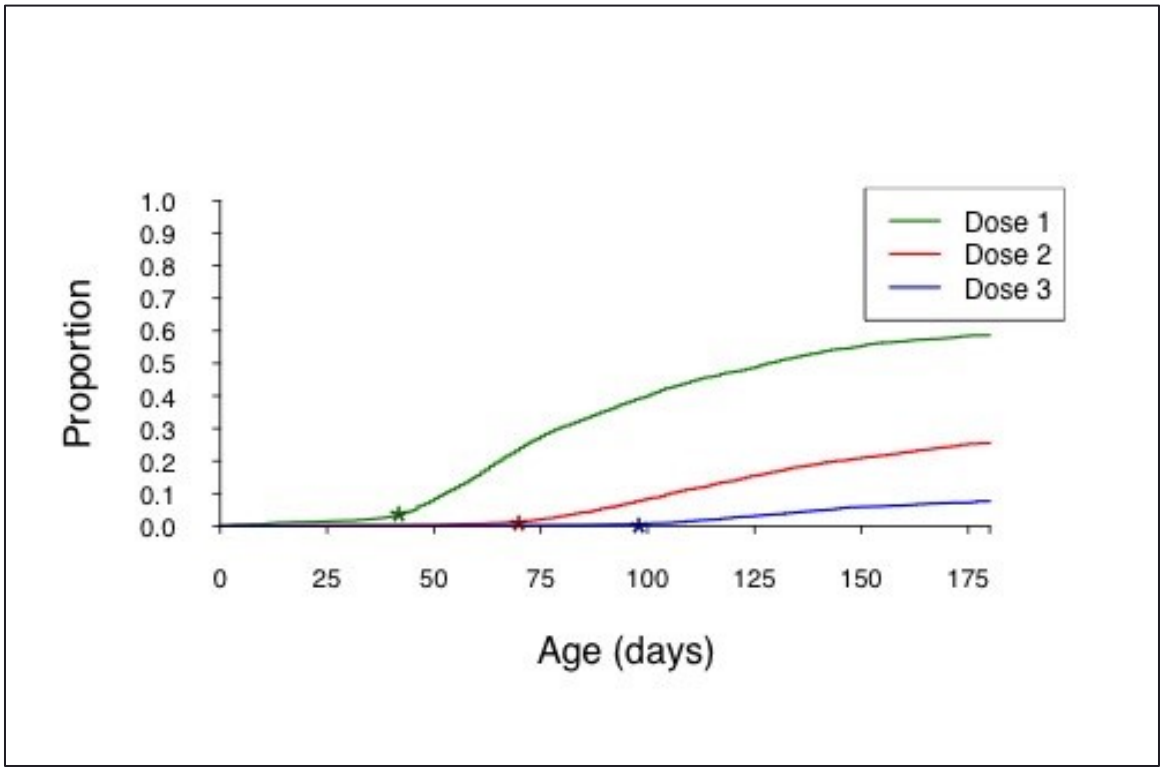
Conclusion

We found substantial delay in receipt of the primary pertussis vaccination series in a prospective population-based cohort in Sarlahi District, Nepal. Nepal national immunization coverage figures do not fully capture the excess pertussis risk attributable to delays in vaccination. Timeliness of routine childhood immunization should be emphasized to maintain the low pertussis incidence. Groups at higher risk for non-vaccination in Nepal including children born SGA, mothers who delay initiation of breastfeeding, and mothers of Madeshi ethnicity should be targeted for immunization timeliness interventions. Age appropriate vaccination indicators should be considered as another metric of an immunization program's success.



*Excludes infants observed only between ages 1-97 days.

FIGURE 3.1 – PERTUSSIS VACCINE DOSE COVERAGE



*Stars indicate the recommended age for each vaccine dose

FIGURE 3.2 – KAPLAN-MEIER TIME TO PERTUSSIS IMMUNIZATION

Table 3.1 - Risk Factors for Time to First Pertussis Vaccination
Cox Proportional Hazard Model

Risk Factor			Unadjusted			Adjusted		
	No	%	HR[1]	95% CI[2]	p-value[3]	HR	95% CI	p-value
Sex								
Female	1601	47%						
Male	1795	53%	1.07	0.975 - 1.17	0.15	1.04	0.930 - 1.15	0.53
Gestational Age[4]								
Term	2983	88%						
Pre-Term	408	12%	1.12	0.965 - 1.29	0.14	1.18	0.990 - 1.42	0.065
Birthweight[5]								
Normal	2020	76%						
Low Birthweight	645	24%	1.13	0.999 - 1.28	0.053			
Small-for-Gestational Age[6]								
non-SGA	1413	53%						
SGA	1271	47%	1.12	1.02 - 1.25	**0.025**	1.13	1.01 - 1.26	**0.038**
Breastfeeding								
Breastfed <1 hour	1170	35%						
Non-breastfed 1st hour	2121	64%	1.18	1.07 - 1.29	**<0.001**	1.13	1.01 - 1.27	**0.033**
Literacy								
Literate	1816	61%						
Illiterate	1173	39%	1.11	1.00 - 1.22	**0.050**	1.06	0.926 - 1.20	0.42
Parity								
Non-first pregnancy	1769	59%						
First pregnancy	1244	41%	1.06	0.961 - 1.17	0.24	1.08	0.962 - 1.22	0.19
Ethnicity								
Pahdai	1778	58%						
Madeshi	1272	42%	1.35	1.23 - 1.50	**<0.0001**	1.20	1.05 - 1.36	**0.0066**
SES[7]								
Lower vs. higher	3051		1.03 [9]	0.984 - 1.07	0.22	0.99	0.939 - 1.05	0.75
Crowding[8]								
Uncrowded	1966	64%						
Crowded	1086	36%	1.12	1.01 - 1.24	**0.028**			
Children under 15 years								
≤3 children	2292	75%						
>3 children	760	25%	1.18	1.05 - 1.32	**0.0055**	1.08	0.943 - 1.23	0.27
Children under 5 years								
≤ 1 child	2094	69%						
>1 child	958	31%	1.05	0.942 - 1.16	0.40			

1 Hazard Ratio; interpretation: ratio of the hazard of being unvaccinated in risk group compared to the reference group
2 95% confidence interval
3 P-values calculated from the Wald test of the maximum likelihood estimate (MLE) of the coefficient
4 Gestational age: Preterm (<37 weeks), Term (≥37 weeks)
5 Birthweight: Low birthweight (<2500 grams), Normal (≥2500 grams)
6 Small-for-gestational age: SGA (< than the 10%), non-SGA (≥ than the 10%)
7 Socioeconomic status (SES): Average of 24 SES measures categorized into quartiles and modeled as a continuous variable (1-4)
8 Crowding: Crowded (≥10 persons living in household), Uncrowded (<10 persons living in household)
9 Interpretation example: hazard of being unvaccinated in the bottom quartile compared to 2nd lowest quartile

Chapter 3 References

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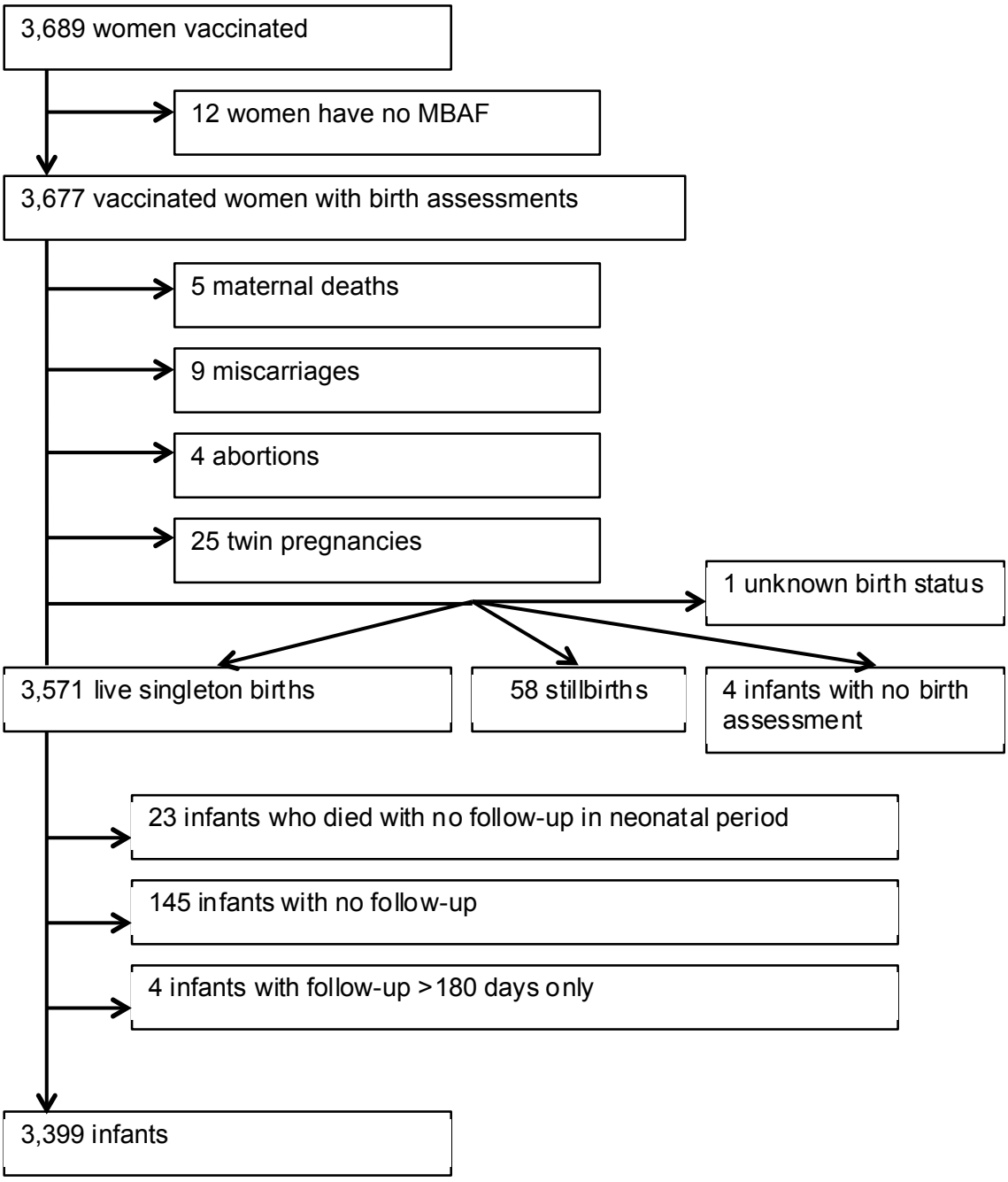
Chapter 3 Appendices

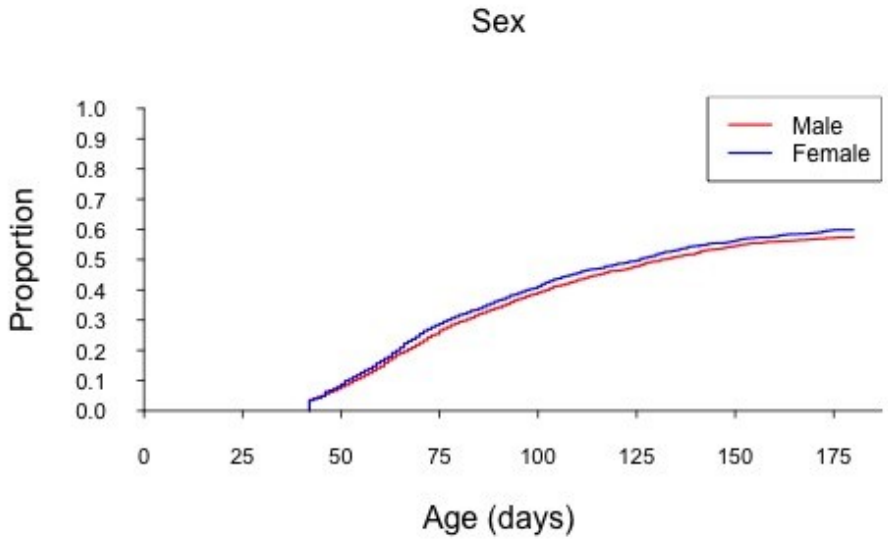
Appendix 3.1

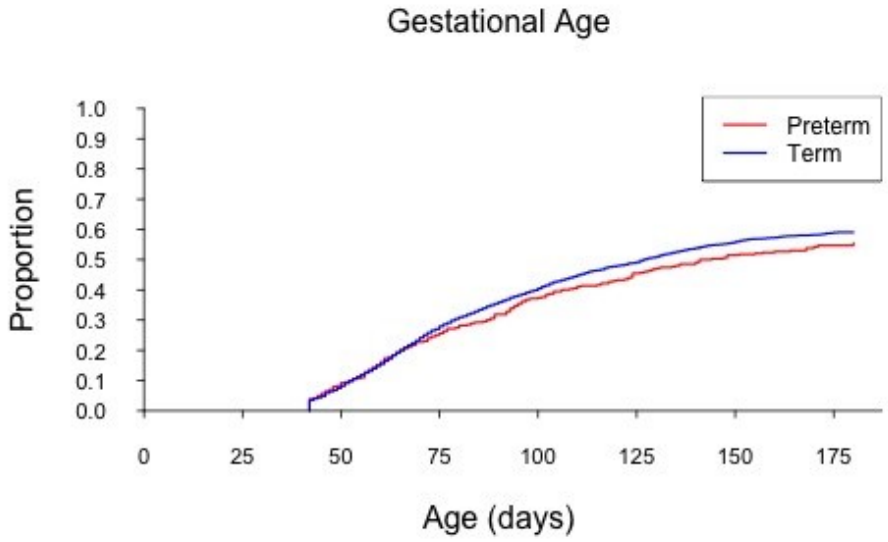
Population Selection Chart

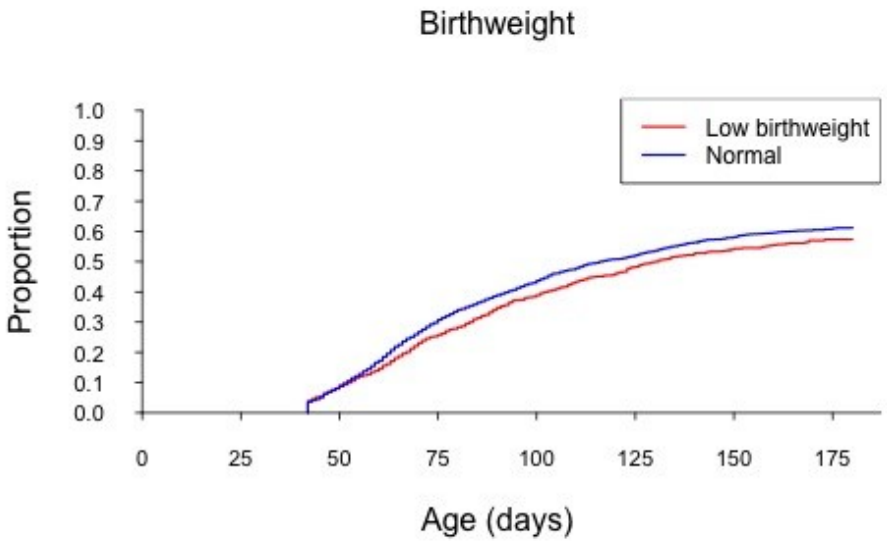


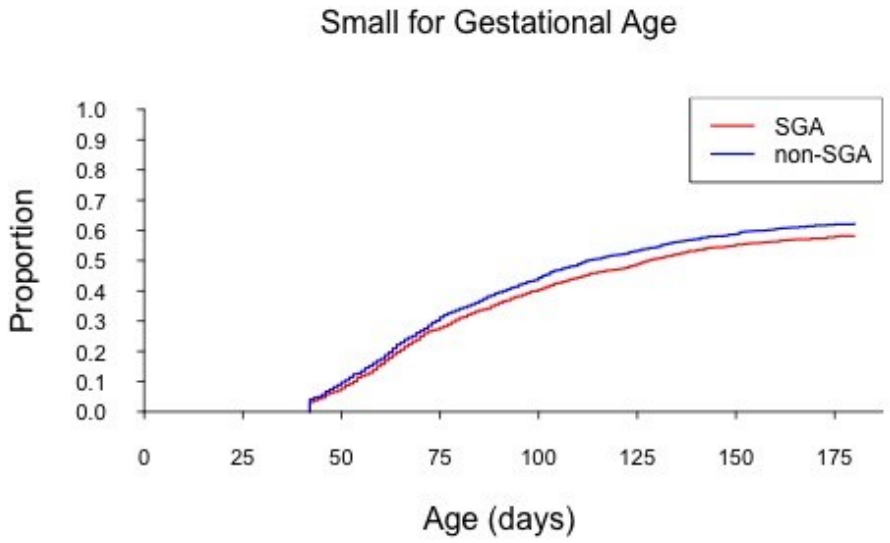
Appendix 3.2

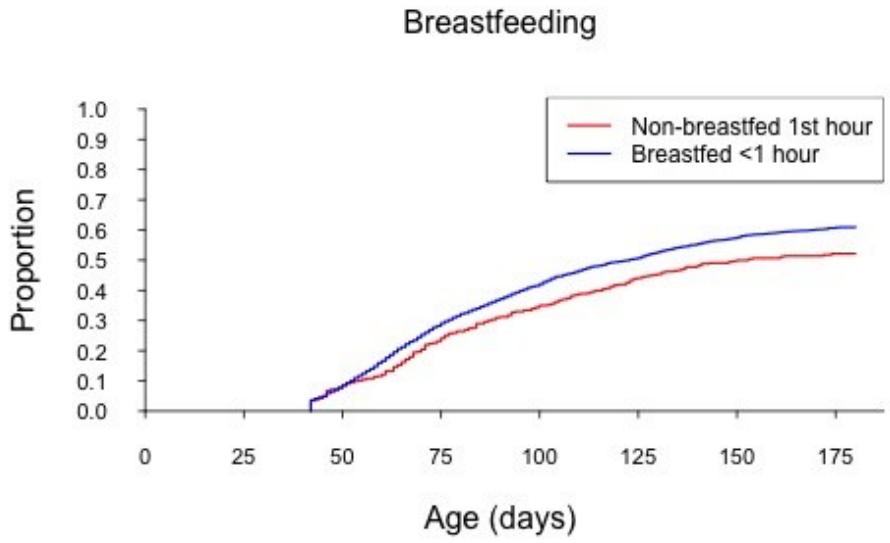
Kaplan-Meier graphs comparing time to first pertussis vaccination by risk group

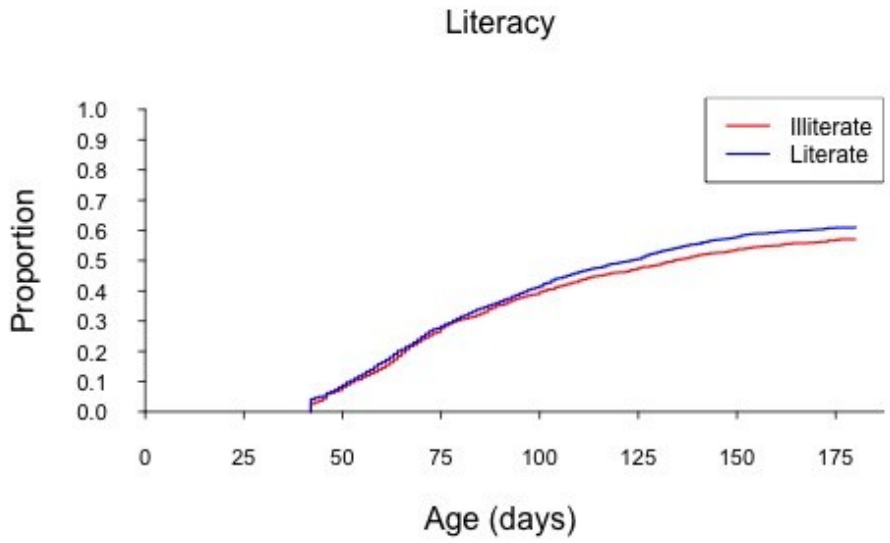


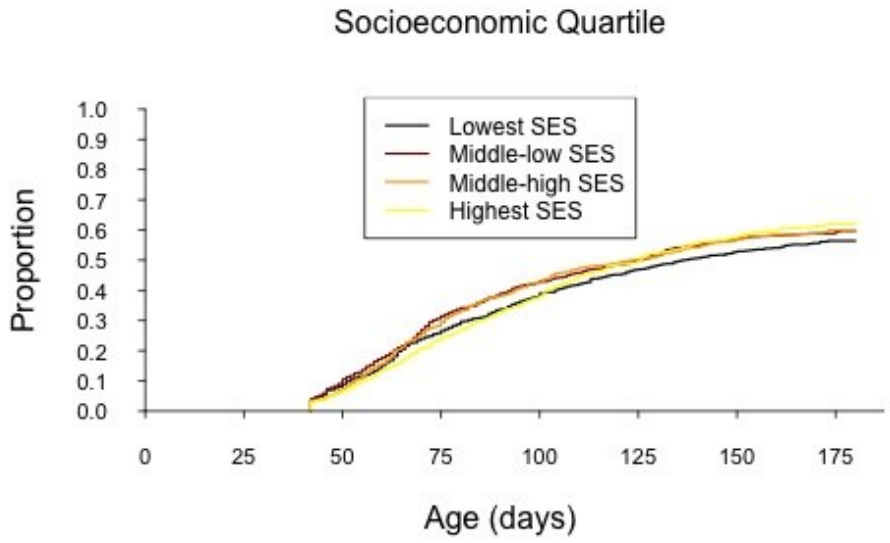


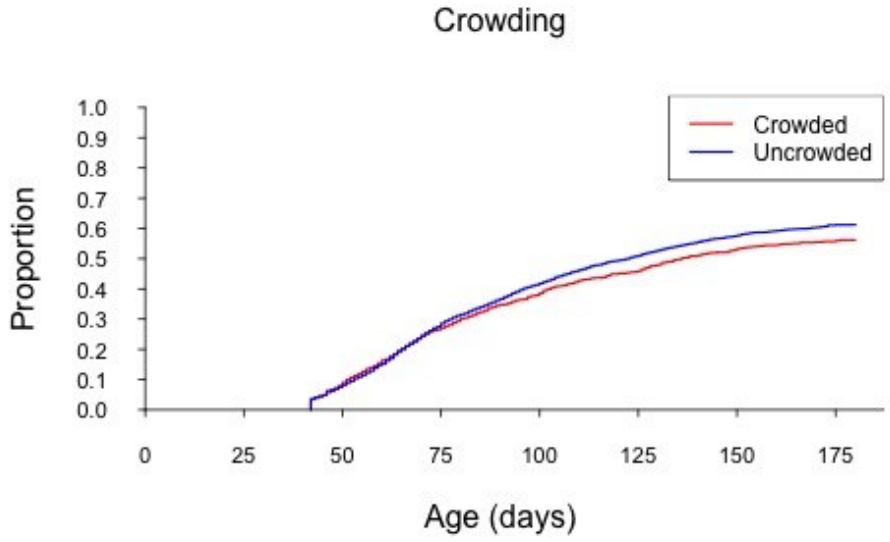

Birthweight
Low birthweight
Normal
Proportion
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
25
50
75
100
125
150
175
Age (days)

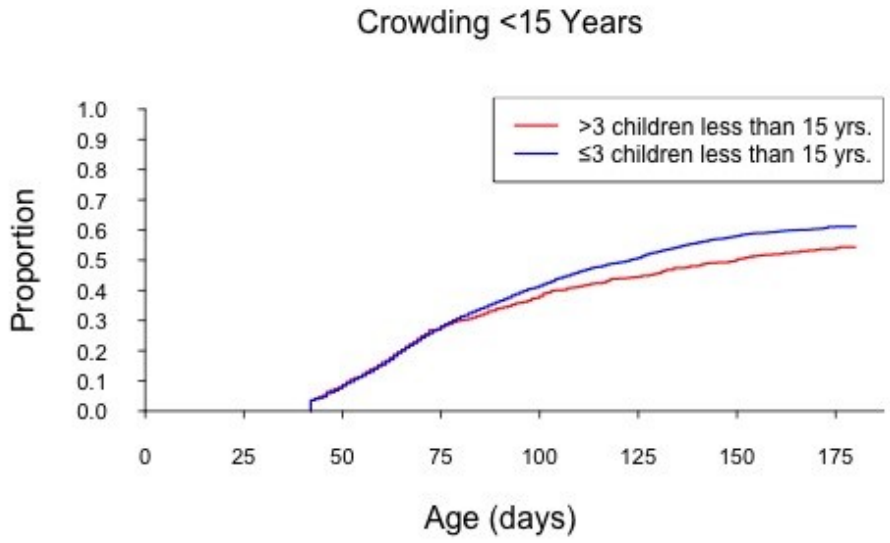

Small for Gestational Age
SGA
non-SGA
Proportion
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
25
50
75
100
125
150
175
Age (days)

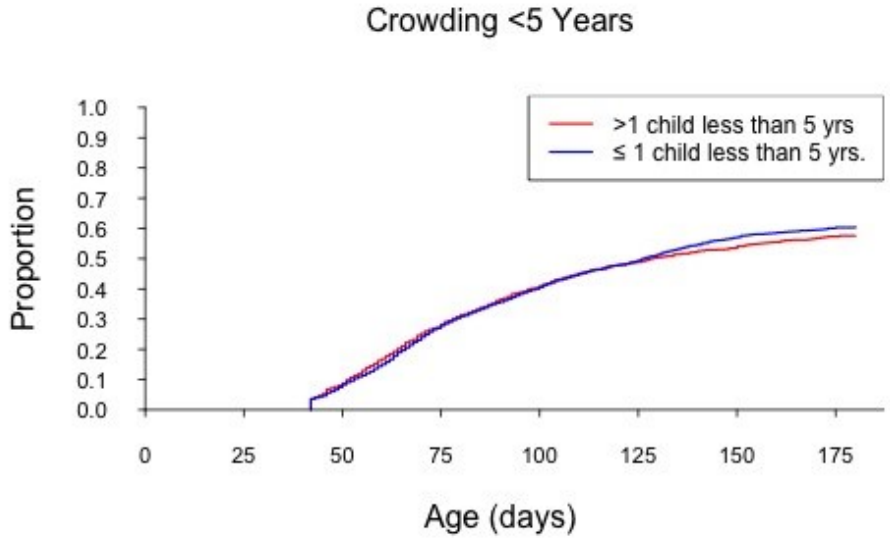

Breastfeeding
Non-breastfed 1st hour
Breastfed <1 hour
Proportion
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
25
50
75
100
125
150
175
Age (days)


Literacy
Illiterate
Literate
Proportion
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
25
50
75
100
125
150
175
Age (days)


Socioeconomic Quartile
Lowest SES
Middle-low SES
Middle-high SES
Highest SES
Proportion
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
25
50
75
100
125
150
175
Age (days)


Crowding
Crowded
Uncrowded
Proportion
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
25
50
75
100
125
150
175
Age (days)


Crowding <15 Years
>3 children less than 15 yrs.
≤3 children less than 15 yrs.
Proportion
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
25
50
75
100
125
150
175
Age (days)


Crowding <5 Years
>1 child less than 5 yrs
≤ 1 child less than 5 yrs.
Proportion
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
25
50
75
100
125
150
175
Age (days)

Appendix 3.3

Schoenfeld residuals to test cox proportional hazards proportionality assumption

Schoenfelds residuals	
Variable	**p**
Sex	0.556
Gestational Age	0.567
Birthweight	0.487
SGA	0.755
Breastfeeding	0.362
Literacy	0.506
Parity	**0.0397**
Ethnicity	0.487
SES	**0.00135**
Crowding	0.0832
Children <15	**0.0148**
Children <5	**0.0312**

Appendix 3.4

Risk Factors for Time to Second Pertussis Vaccination
Cox Proportional Hazard Model

Risk Factor			Unadjusted			Adjusted		
	No	%	HR[1]	95% CI[2]	p-value[3]	HR	95% CI	p-value
Sex								
Female	1600	47%						
Male	1794	53%	0.97	0.843 - 1.12	0.71	0.93	0.786 - 1.10	0.38
Gestational Age[4]								
Term	2982	88%						
Pre-Term	407	12%	0.98	0.791 - 1.22	0.88	1.18	0.90 - 1.56	0.23
Birthweight[5]								
Normal	2019	76%						
Low Birthweight	644	24%	1.19	0.978 - 1.44	0.082			
Small-for-Gestational Age[6]								
non-SGA	1412	53%						
SGA	1270	47%	1.25	1.06 - 1.46	**0.0070**	1.33	1.114 - 1.59	**0.002**
Breastfeeding								
Breastfed <1 hour	1169	36%						
Non-breastfed 1st hour	2120	65%	1.16	1.00 - 1.35	**0.047**	1.12	0.939 - 1.33	0.21
Literacy								
Literate	1815	61%						
Illiterate	1172	39%	1.05	0.90 - 1.23	0.51	1.11	0.907 - 1.37	0.31
Parity								
Non-first pregnancy	1767	59%						
First pregnancy	1244	41%	1.09	0.93 - 1.27	0.30	1.07	0.887 - 1.28	0.50
Ethnicity								
Pahdai	1777	58%						
Madeshi	1271	42%	1.22	1.04 - 1.42	**0.014**	1.11	0.91 - 1.36	0.29
SES[7]								
Lower vs. higher	3049		1.00 [9]	0.932 - 1.07	0.929	0.99	0.912 - 1.08	0.83
Crowding[8]								
Uncrowded	1964	64%						
Crowded	1086	36%	1.20	1.02 - 1.41	**0.028**			
Children under 15 years								
≤3 children	2290	75%						
>3 children	760	25%	1.11	0.93 - 1.32	0.26	1.17	0.948 - 1.44	0.15
Children under 5 years								
≤ 1 child	2092	69%						
>1 child	958	31%	1.04	0.886 - 1.22	0.62			

1 Hazard Ratio; interpretation: ratio of the hazard of being unvaccinated in risk group compared to the reference group
2 95% confidence interval
3 P-values calculated from the Wald test of the maximum likelihood estimate (MLE) of the coefficient
4 Gestational age: Preterm (<37 weeks), Term (≥37 weeks)
5 Birthweight: Low birthweight (<2500 grams), Normal (≥2500 grams)
6 Small-for-gestational age: SGA (< than the 10%), non-SGA (≥ than the 10%)
7 Socioeconomic status (SES): Average of 24 SES measures categorized into quartiles and modeled as a continuous variable (1-4)
8 Crowding: Crowded (≥10 persons living in household), Uncrowded (<10 persons living in household)
9 Interpretation example: hazard of being unvaccinated in the bottom quartile compared to 2nd lowest quartile

Appendix 3.5

Risk Factors for Time to Third Pertussis Vaccination
Cox Proportional Hazard Model

Risk Factor			Unadjusted			Adjusted		
	No	%	HR[1]	95% CI[2]	p-value[3]	HR	95% CI	p-value
Sex								
Female	1599	47%						
Male	1793	53%	0.86	0.654 - 1.12	0.26	0.86	0.628 - 1.18	0.35
Gestational Age[4]								
Term	2981	88%						
Pre-Term	406	12%	0.82	0.564 - 1.21	0.32	0.89	0.56 - 1.41	0.62
Birthweight[5]								
Normal	2018	76%						
Low Birthweight	643	24%	1.34	0.922 - 1.95	0.13			
Small-for-Gestational Age[6]								
non-SGA	1410	53%						
SGA	1270	47%	1.45	1.07 - 1.96	**0.016**	1.40	1.000 - 1.96	**0.050**
Breastfeeding								
Breastfed <1 hour	1169	35%						
Non-breastfed 1st hour	2118	64%	1.43	1.09 - 1.88	**0.009**	1.49	1.076 - 2.05	**0.016**
Literacy								
Literate	1814	61%						
Illiterate	1171	39%	0.96	0.72 - 1.28	0.76	1.27	0.871 - 1.85	0.22
Parity								
Non-first pregnancy	1765	59%						
First pregnancy	1244	41%	1.05	0.786 - 1.40	0.74	0.97	0.691 - 1.37	0.88
Ethnicity								
Pahdai	1776	58%						
Madeshi	1270	42%	0.99	0.74 - 1.31	0.92	0.89	0.62 - 1.29	0.55
SES[7]								
Lower vs. higher	3047		0.88 [9]	0.776 - 1.00	0.05	0.86	0.738 - 1.01	0.068
Crowding[8]								
Uncrowded	1963	64%						
Crowded	1085	36%	1.17	0.87 - 1.58	0.31			
Children under 15 years								
≤3 children	2289	75%						
>3 children	759	25%	1.29	0.92 - 1.82	0.15	1.55	1.015 - 2.37	**0.043**
Children under 5 years								
≤ 1 child	2092	69%						
>1 child	956	31%	1.04	0.771 - 1.41	0.79			

1 Hazard Ratio; interpretation: ratio of the hazard of being unvaccinated in risk group compared to the reference group
2 95% confidence interval
3 P-values calculated from the Wald test of the maximum likelihood estimate (MLE) of the coefficient
4 Gestational age: Preterm (<37 weeks), Term (≥37 weeks)
5 Birthweight: Low birthweight (<2500 grams), Normal (≥2500 grams)
6 Small-for-gestational age: SGA (< than the 10%), non-SGA (≥ than the 10%)
7 Socioeconomic status (SES): Average of 24 SES measures categorized into quartiles and modeled as a continuous variable (1-4)
8 Crowding: Crowded (≥10 persons living in household), Uncrowded (<10 persons living in household)
9 Interpretation example: hazard of being unvaccinated in the bottom quartile compared to 2nd lowest quartile

Appendix 3.6

Risk Factors for No Pertussis Vaccination at 6 Months
Logistic Regression Model

Risk Factor	>0 Doses at 6 Months		Unadjusted			Adjusted		
	No	%	OR[1]	95% CI[2]	p-value[3]	OR	95% CI	p-value
Sex								
Female	858	58%						
Male	935	56%	1.09	0.95 - 1.26	0.21	1.04	0.874 - 1.24	0.66
Gestational Age[4]								
Term	1584	58%						
Pre-Term	205	54%	1.17	0.942 - 1.45	0.15	1.28	0.98 - 1.68	0.07
Birthweight[5]								
Normal	1122	60%						
Low Birthweight	330	56%	1.16	0.959 - 1.40	0.13			
Small-for-Gestational Age[6]								
non-SGA	803	61%						
SGA	659	57%	1.18	1.00 - 1.38	**0.045**	1.20	0.996 - 1.43	0.055
Breastfeeding								
Breastfed <1 hour	675	61%						
Non-breastfed 1st hour	1090	56%	1.24	1.07 - 1.44	**0.006**	1.19	0.99 - 1.43	0.064
Literacy								
Literate	1007	60%						
Illiterate	603	56%	1.16	1.00 - 1.36	0.055	1.01	0.823 - 1.25	0.90
Parity								
Non-first pregnancy	968	59%						
First pregnancy	656	57%	1.05	0.898 - 1.22	0.56	1.04	0.863 - 1.26	0.65
Ethnicity								
Pahdai	1049	63%						
Madeshi	593	51%	1.59	1.37 - 1.85	**<0.0001**	1.30	1.07 - 1.59	**0.01**
SES[7]								
Lower vs. higher	2826		1.06 [9]	0.995 - 1.14	0.07	1.03	0.946 - 1.13	0.47
Crowding[8]								
Uncrowded	1096	60%						
Crowded	547	55%	1.22	1.05 - 1.43	**0.011**			
Children under 15 years								
≤3 children	1265	60%						
>3 children	378	53%	1.30	1.10 - 1.55	**0.002**	1.15	0.933 - 1.41	0.19
Children under 5 years								
≤ 1 child	1131	59%						
>1 child	512	57%	1.09	0.932 - 1.28	0.27			

1 Odds Ratio; interpretation: ratio of the odds of being unvaccinated in risk group compared to odds of being unvaccinated in the reference group
2 95% confidence interval
3 P-values calculated from Wald z-statistic
4 Gestational age: Preterm (<37 weeks), Term (≥37 weeks)
5 Birthweight: Low birthweight (<2500 grams), Normal (≥2500 grams)
6 Small-for-gestational age: SGA (< than the 10%), non-SGA (≥ than the 10%)
7 Socioeconomic status (SES): Average of 24 SES measures categorized into quartiles and modeled as a continuous variable (1-4)
8 Crowding: Crowded (≥10 persons living in household), Uncrowded (<10 persons living in household)
9 Interpretation example: odds of being unvaccinated in the bottom quartile compared to unvaccinated in the 2nd lowest quartile

Appendix 3.7

Risk Factors for One or Less Pertussis Vaccination Doses 6 Months
Logistic Regression Model

Risk Factor	2 or 3 Doses at 6 months		Unadjusted			Adjusted		
	No	%	OR[1]	95% CI[2]	p-value[3]	OR	95% CI	p-value
Sex								
Female	345	23%						
Male	401	24%	0.97	0.82 - 1.14	0.69	0.93	0.766 - 1.13	0.47
Gestational Age[4]								
Term	652	24%						
Pre-Term	92	24%	0.98	0.764 - 1.26	0.86	1.20	0.88 - 1.66	0.25
Birthweight[5]								
Normal	487	26%						
Low Birthweight	133	23%	1.20	0.963 - 1.49	0.11			
Small-for-Gestational Age[6]								
non-SGA	360	27%						
SGA	262	23%	1.28	1.07 - 1.54	**0.008**	1.38	1.125 - 1.70	**0.002**
Breastfeeding								
Breastfed <1 hour	287	26%						
Non-breastfed 1st hour	450	23%	1.17	0.98 - 1.39	0.074	1.12	0.92 - 1.37	0.27
Literacy								
Literate	417	25%						
Illiterate	255	24%	1.06	0.89 - 1.27	0.54	1.12	0.887 - 1.42	0.34
Parity								
Non-first pregnancy	414	25%						
First pregnancy	262	23%	1.12	0.94 - 1.34	0.20	1.09	0.882 - 1.35	0.42
Ethnicity								
Pahdai	433	26%						
Madeshi	251	22%	1.26	1.05 - 1.50	**0.01**	1.16	0.92 - 1.45	0.21
SES[7]								
Lower vs. higher	2826		1.00 [9]	0.927 - 1.08	0.97	1.00	0.905 - 1.10	0.98
Crowding[8]								
Uncrowded	468	26%						
Crowded	216	22%	1.24	1.03 - 1.49	**0.02**			
Children under 15 years								
≤3 children	521	25%						
>3 children	163	23%	1.10	0.90 - 1.34	0.37	1.17	0.924 - 1.49	0.19
Children under 5 years								
≤ 1 child	468	24%						
>1 child	216	24%	1.02	0.852 - 1.23	0.80			

1 Odds Ratio; interpretation: ratio of the odds of being vaccinated with less than 2 doses in risk group compared to odds of being vaccinated with less than 2 doses in the reference group
2 95% confidence interval
3 P-values calculated from Wald z-statistic
4 Gestational age: Preterm (<37 weeks), Term (≥37 weeks)
5 Birthweight: Low birthweight (<2500 grams), Normal (≥2500 grams)
6 Small-for-gestational age: SGA (< than the 10%), non-SGA (≥ than the 10%)
7 Socioeconomic status (SES): Average of 24 SES measures categorized into quartiles and modeled as a continuous variable (1-4)
8 Crowding: Crowded (≥10 persons living in household), Uncrowded (<10 persons living in household)
9 Interpretation example: odds of being vaccinated with less than 2 doses in the bottom quartile compared to being vaccinated with less than 2 doses in the 2nd lowest quartile

Appendix 3.8

Risk Factors for Two or Less Pertussis Vaccination Doses 6 Months
Logistic Regression Model

Risk Factor	3 Doses at 6 months		Unadjusted			Adjusted		
	No	%	OR[1]	95% CI[2]	p-value[3]	OR	95% CI	p-value
Sex								
Female	93	6%						
Male	123	7%	0.85	0.639 - 1.12	0.24	0.86	0.618 - 1.19	0.37
Gestational Age[4]								
Term	185	7%						
Pre-Term	31	8%	0.82	0.556 - 1.23	0.31	0.87	0.55 - 1.44	0.58
Birthweight[5]								
Normal	145	8%						
Low Birthweight	34	6%	1.36	0.937 - 2.03	0.12			
Small-for-Gestational Age[6]								
non-SGA	111	8%						
SGA	68	6%	1.47	1.08 - 2.02	**0.015**	1.41	0.999 - 2.02	0.05
Breastfeeding								
Breastfed <1 hour	94	9%						
Non-breastfed 1st hour	117	6%	1.46	1.10 - 1.93	**0.009**	1.50	1.07 - 2.11	**0.02**
Literacy								
Literate	116	7%						
Illiterate	77	7%	0.96	0.71 - 1.30	0.78	1.29	0.875 - 1.91	0.20
Parity								
Non-first pregnancy	118	7%						
First pregnancy	76	7%	1.08	0.801 - 1.46	0.62	1.00	0.703 - 1.44	0.98
Ethnicity								
Pahdai	116	7%						
Madeshi	80	7%	1.00	0.75 - 1.35	0.999	0.91	0.62 - 1.33	0.62
SES[7]								
Lower vs. higher	2826		0.87 [9]	0.765 - 0.997	**0.046**	0.86	0.726 - 1.01	0.07
Crowding[8]								
Uncrowded	134	7%						
Crowded	62	6%	1.19	0.88 - 1.63	0.28			
Children under 15 years								
≤3 children	155	7%						
>3 children	41	6%	1.29	0.91 - 1.86	0.16	1.55	1.016 - 2.44	0.05
Children under 5 years								
≤ 1 child	134	7%						
>1 child	62	7%	1.02	0.748 - 1.40	0.92			

1 Odds Ratio; interpretation: ratio of the odds of being not fully vaccinated (<3 doses) in risk group compared to odds of being not fully vaccinated in the reference group
2 95% confidence interval
3 P-values calculated from Wald z-statistic
4 Gestational age: Preterm (<37 weeks), Term (≥37 weeks)
5 Birthweight: Low birthweight (<2500 grams), Normal (≥2500 grams)
6 Small-for-gestational age: SGA (< than the 10%), non-SGA (≥ than the 10%)
7 Socioeconomic status (SES): Average of 24 SES measures categorized into quartiles and modeled as a continuous variable (1-4)
8 Crowding: Crowded (≥10 persons living in household), Uncrowded (<10 persons living in household)
9 Interpretation example: odds of being not fully vaccinated (<3 doses) in the bottom quartile compared to not being fully vaccinated in the 2nd lowest quartile

CHAPTER 4

Pertussis Incidence and Risk Factors in Infants <6 Months in Sarlahi, Nepal: A Population-Based Prospective Cohort

Authors

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Abstract

Background: Pertussis is estimated to cause 2% of childhood deaths globally and is a growing public health problem in developed countries despite high vaccination coverage. Infants are at greatest risk of morbidity and mortality. Maternal vaccination during pregnancy may be effective to prevent pertussis in young infants but population-based estimates of disease burden in infants, particularly in low-income countries are lacking.

Objective: To estimate the incidence of pertussis in infants <6 months of age in Sarlahi District, Nepal.

Design/Methods: Nested within a larger randomized controlled trial of influenza vaccination during pregnancy, infants were visited weekly from birth through six months to assess respiratory illness in the prior week. If any respiratory symptoms had occurred, a nasal swab was collected and tested with a multi-target pertussis PCR assay. Infants observed between August 17, 2011 and August 16, 2013 were included in the analysis.

Results: The incidence of PCR-confirmed *Bordetella pertussis* and *Bordetella parapertussis* was 5.2 cases per 1000 infant-years (95% CI, 2.1 – 10.7) and 3.0 cases per 1000 infant-years (95% CI, 0.81 – 7.6) respectively, in a cohort of 3,235 infants with at least one-week of follow-up.

Conclusions: Population-based active home surveillance for respiratory illness found a low risk for pertussis among infants in rural Nepal. Nepal's immunization program, which includes a childhood whole cell pertussis vaccine, appears to be working in controlling pertussis in infants.

Introduction

A resurgence of pertussis across age groups has occurred in several countries in recent years[1,2]. Middle and high-income countries, using an acellular pertussis vaccine for the primary vaccination series, have been particularly affected[3]. Moreover, age groups experiencing the greatest increase include infants and adolescents[4]. Several factors are thought to contribute to the increased pertussis levels, which include rapid waning immunity from those primarily or exclusively vaccinated with acellular vaccines versus whole cell vaccines[5,6], genetic adaption of *Bordetella pertussis*[7], vaccination delay or refusal[8,9], improved surveillance and laboratory capabilities[3], and overall increased awareness of the continuing circulation of *B. pertussis*[2].

As infants experience the greatest morbidity and mortality from pertussis some countries experiencing epidemic pertussis, namely the U.S. and U.K., now recommend pertussis immunization in pregnancy and vaccination of close contacts[10,11]. The purpose of this strategy is to protect the youngest infants from pertussis before they can be vaccinated themselves[12]. Global estimates of pertussis place the highest childhood burden in Southeast Asia[13]. Maternal pertussis vaccination may be of public health interest here and in similar settings, as a measure to protect infants such as currently exists for tetanus toxoid vaccine. However, only one population-based estimate of pertussis in infants from birth has been conducted (Senegal)[14] and surveillance and laboratory capabilities in Asia are lacking[15,16]. Further, the World Health Organization (WHO) recently recommend-

ed that countries using whole cell pertussis vaccines continue to do so in light of recent data indicating that acellular pertussis vaccines are less effective than whole cell pertussis vaccines[3]. Population-based data are needed, especially in low-income settings, to provide a more accurate estimate of the burden of pertussis in infants to inform childhood and maternal immunization policies. This study was a two-year prospective cohort following infants in their homes to monitor for pertussis from birth to age 6 months. The objective of this study was to provide the first population-based estimate of laboratory confirmed pertussis incidence in infants <6 months in Asia.

Methods

Settings and population

The setting of the study was Sarlahi District, located in the central terai (low lying plains) region of Nepal. The study was nested within a randomized controlled trial of maternal influenza vaccination during pregnancy. At the start of the trial, prevalent pregnancies were identified through a survey census of all households in the catchment area. For the duration of the trial field workers visited all households in the community where married women (15 – 40 years) resided every 5 weeks for surveillance of incident pregnancies. Once a pregnancy was identified women were asked for their consent to participate in the trial. From April 25, 2011 through September 9, 2013 women between 17-34 weeks gestation were randomized and vaccinated with either an influenza vaccine or placebo. All participants received ancillary benefits, which included a 90-day supply of iron-folic acid tablets, deworming medicine (single dose of albendazole), clean birthing kit, chlorhexidine ointment for umbilical cord care, a tetanus toxoid vaccine, if indicated, and health education messages, in addition to antenatal services according to the local standard of care. The study was a population-based prospective cohort of infants followed from birth through 6 months post-partum. Approval for the study was obtained from the Johns Hopkins Bloomberg School of Public Health Institutional Review Board and the local ethical review board (Institute of Medicine at Tribhuvan University/Nepal Health Research Council).

Data collection

At baseline information was collected on household structure, socioeconomic status, and demographics. At study enrollment, date of last menstrual period and pregnancy history data were collected. As soon as possible after delivery the mother and infant were visited to collect detailed birth information including infant weight and breastfeeding status. From birth through 6 months postpartum infants were visited weekly by a field worker, who recorded any infant respiratory symptoms in the past 7 days. If an infant had any of the following symptoms a mid-nasal swab was collected: fever, cough, wheeze, difficulty breathing, or ear infection. Starting on August 17, 2012 new symptoms more specific for pertussis were added to the weekly morbidity visit: apnea, cyanosis, cough with vomit, or whoop/whooping cough. In addition to these signs, mothers were asked which, if any, vaccinations were received in the past 7 days. At 6 months child anthropometry measures were taken for weight and length.

Laboratory Assays

Real-time PCR testing was conducted at the University of Washington's Molecular Virology Laboratory according to previously published methods[17]. Two-target PCR was used to assess the presence of three Bordetella species: *B. pertussis, B. parapertussis, and B. bronchiseptica*. The two independent pertussis sequence targets amplified were chromosomal repeated insertion sequence IS481 (IS) and the polymorphic pertussis toxin ptxA promoter region (PT). There were 2 sets of primers for PT to accommodate small differences (2 bases) between pertussis strains (used in combination for one PCR reaction).

The pertussis PCR assay used fluorescence resonance energy transfer SYBR green chemistry. The PCR cycle for PT amplification was as follows: 95°C for 3 minutes, followed by 45 cycles of 95°C for 10 seconds and 71°C for 45 seconds. The PCR cycle for IS amplification was as follows: 95°C for 2 minutes, followed by 45 cycles of 94°C for 30 seconds, 68°C for 30 seconds, and 72°C for 30 seconds and then 72°C for 5 minutes for extension. After completion of the 45th replication cycle, the melting points of the amplicons were measured in an iCycler (Bio-Rad).

A sample was interpreted as positive when the targets described in Appendix 4.1 had a melting temperature within the acceptable range and a Ct ≤42. A sample was negative if none of the targets tested positive or a single positive target was not reproducible.

Analytic Dataset

Infants were included in this analysis if they were followed for any length (0 to 180 days) during a 2 year-period from August 17, 2011 to August 16, 2013. [Complete data from the trial is pending]. See Appendix 4.2 – Vaccine Delay Sample for detailed sample selection procedures. Of 3,689 women vaccinated, 12 were missing a maternal delivery assessment (due to incomplete data entry at time of analysis) leaving 3,677 women with delivery assessment information. There were 5 maternal deaths, 9 miscarriages, and 4 abortions. Twenty-five twin pregnancies were excluded from the analysis. Of the 3,634 singleton births, there were 3,571 live births, 58 stillbirths, and 5 had missing birth information. Of the

3,571 live singleton births, 336 were born outside of the specified pertussis cohort period (August 2011 through August 2013). The final dataset consists of 3,325 infants with at least one follow-up visit during the first 6 months.

At baseline, data on household structure was gathered, including age and sex of all household members. At enrollment women reported their literacy status (binary), years of education and pregnancy history. The field workers identified their ethnicity (Pahadi or Madeshi) from names and observation. For parity analysis women were categorized as nulliparous or multiparous. Responses to twenty-five questions about household construction, water and sanitation, and household assets were used to develop a construct to measure the socioeconomic status (SES) of households. Binary variables for each of the 25 questions were created and a score from 0 to 25 was obtained. A percentage was created from this score. If some variables were missing responses, percentages were created using the number of responses available as the denominator. The percentages were then divided into SES quartiles for analysis.

Gestational age was measured using a woman's report of date of last menstrual period during pregnancy surveillance (an average of 3-4 weeks recall). Birthweight was collected as soon as possible after birth using a digital scale [Tanita model BD-585, precision to nearest 10g]. Birthweights collected >72 hours after birth were excluded from the analysis of birthweight. Small for gestational age was calculated using the sex-specific 3rd percentile cut-off described by Oken[18]. Women were asked within how many hours of birth breastfeeding

was initiated (if any). Binary breastfeeding categories were created with women initiating breastfeeding within 1 hour compared to those initiating >1 hour post-delivery. Anthropometry measures were calculated from the 6-month weight and length measurements. The z-scores for underweight (weight for age), stunting (length for age), and wasting (weight for length) were calculated using the WHO Child Growth Standards from the *igrowup* Stata package.

Statistical Analysis

Incidence was calculated as the number of pertussis cases per 1000 infant-years at risk. 95% confidence intervals were constructed using Poisson exact confidence intervals. Descriptions of pertussis episodes and of maternal, household, and infant characteristics of pertussis cases are presented. Characteristics of all non-pertussis respiratory episodes were examined in comparison to pertussis episodes. T-tests were used to compare associations with continuous predictors and Fisher's exact test was used to compare categorical associations since the number of pertussis cases was small. Characteristics of pertussis cases were compared to non-pertussis cases. Continuous predictors were transformed to dichotomous factors to calculate incidence rate ratios for pertussis risk. Statistical significance was set at $p<0.05$ for all testing. All statistical analyses were conducted in Stata/SE 13.1.

Results

3,235 infants had 3,605 episodes of respiratory illness [See Respiratory Illness Definitions Text Box] over a two-year period. The mean incidence of respiratory illness was 2.81 episodes per infant-year (95% CI: 2.72 – 2.91) with a range of 0 to 8 episodes per child during a maximum of 6 months follow-up. Episode duration averaged 4.74 days (95% CI: 4.62 – 4.87). 424 episodes were not matched to a nasal swab and 323 nasal swabs were not tested by PCR for pertussis. 2,924 episodes were matched to 1 or more pertussis-tested nasal swabs.

Respiratory Illness Definitions

Year 1 - Experienced at least one of the following symptoms: cough, wheeze, difficulty breathing, fever or ear infection.

Year 2 – Any of the symptoms from year 1 or any of the following: cyanosis, apnea, cough with vomit, whooping cough/whoop.

Seven cases of *Bordetella pertussis* were identified from 8 nasal swabs (nasal swabs were positive on two consecutive weeks for one infant) [Tables 4.1A-C]. The incidence of PCR confirmed *B. pertussis* was 5.2 cases per 1000-infant years (95% CI: 2.09 – 10.71). Four cases of *Bordetella parapertussis* were detected with incidence 3.0 cases per 1000 infant-years (95% CI: 0.81 – 7.60) [Tables 4.2A-C]. No cases of *Bordetella bronchiseptica* were identified.

Bordetella pertussis

The average pertussis episode duration was 13 days [Table 4.3]. Mean age of onset of pertussis symptoms was 72 days with a range of 19 to 109 days.

The most common symptoms were fever, cough, and wheeze. None of the infants were reported to have an ear infection, cyanosis, or apnea. We added additional symptoms related to pertussis in year 2 (cyanosis, apnea, cough with vomit, and whoop) however none of those yielded additional nasal swabs beyond those already collected for the year 1 symptom list (fever, cough, difficulty breathing, wheeze, and ear infection). All cases with the exception of one occurred between March and June of 2013. Three of the infants had one pertussis vaccination prior to disease onset although the spacing was short at 5, 16, or 22 days [Table 4.1C]. Comparison of infant characteristics in the population to pertussis cases showed some differences although none were statistically significant [Table 4.4]. Given that our study does not have adequate power due to low number of pertussis cases the lack of a statistical association is not evidence of non-association. Episode symptoms and timing were compared between pertussis positive episodes and pertussis negative episodes. Longer episode duration ($p<0.001$) and presence of a whoop ($p=0.047$) were the only statistically significant characteristics associated with a pertussis episode.

Bordetella parapertussis

The average parapertussis episode duration was 4 days [Table 4.2A]. Mean age of onset of symptoms was 49 days with a range of 7 to 71 days. The most common symptoms were cough and wheeze. All parapertussis cases occurred during the first year of follow-up (none in year 2).

Bordetella bronchiseptica

No cases of *B. bronchiseptica* were observed.

Discussion

Low incidence of pertussis and generally mild case presentation was found in infants <6 months in Nepal. To our knowledge this was the first population-based active surveillance of PCR confirmed pertussis in infants in Asia. While truly comparable data are sparse, the acellular pertussis vaccine trials conducted in the 1990s found the average pertussis incidence in the whole cell vaccine groups ranged from 1 to 37 cases per 1000 infant-years[14,19-24]. Our finding of 5 B. pertussis cases per 1000 infant-years was on the lower end of this range. Further, given our highly sensitive case detection method, many of our pertussis cases would likely not have been detected in the previous trials. More stringent respiratory symptom criteria would have lowered our incidence estimate even further. These data support the WHO's recommendation that countries using whole cell pertussis vaccine continue to do so given the pertussis resurgence has not been equally distributed and most outbreaks have been concentrated in countries using the acellular pertussis vaccine[3]. Recent studies also suggest that protection from acellular pertussis vaccine is not as strong or long lasting as that conferred by the whole cell pertussis vaccine[5,6,25-29]. Another contributing factor to the low pertussis incidence observed could be that we conducted the surveillance during a period of low pertussis burden. Pertussis is a cyclical disease, thought to peak every 2 to 4 years, and we may have captured the burden at a low circulation period [6].

We observed almost 90% of our cases in a five-month period between February and June 2013. Only 1 case was observed in 2012 (February) but the case occurred in the same time window observed in 2013. The increase in cases in 2013 could indicate an upward trend of pertussis from a low in 2012. Previous research on pertussis seasonality has been mixed, with studies in different places and time periods demonstrating various periods of peak transmission or no discernable patterns[30-35]. While our data support a seasonal pattern, the numbers observed are too low to be conclusive.

Pertussis symptom duration and severity were on average mild compared to the classic pertussis case presentation. While all cases were multi-target PCR confirmed, only two of the seven cases fulfilled the WHO criteria, which requires a minimum of 2 weeks of cough, whoop, or post-tussive vomiting[36]. One pertussis case had symptoms reported for only 2 days. Studies on pertussis in infants have generally been clinic-based, hospital-based, or in an outbreak which therefore required a certain severity of illness for parents to recognize a need for medical attention[37,44]. These study designs and passive surveillance efforts therefore may have missed milder pertussis cases. Our study, which required only 1 respiratory symptom for a nasal swab to be collected, had increased sensitivity to detect a range of pertussis case presentations. An alternative explanation for the mild cases seen could be an increase in the proportion of mild compared to severe pertussis cases in Nepal. We do not have previous comparable data from Nepal with which to compare our findings.

While cough and wheeze were the most common symptoms, neither were present in all *B. pertussis* cases. During an epidemic period in Washington state (2002-2007) infants <1 year, who had a minimum of 14 days cough plus an additional symptom had the following symptom prevalence: 82% post-tussive emesis, 29% apnea, 26% whoop and 42% cyanosis[37]. A study of U.S. neonates with pertussis showed the symptom prevalence to be 97% for cough, 91% for cyanosis, 58% for apnea, and 3% for fever[38]. In comparison our study found lower or equal symptoms prevalence with the exception of fever (fever prevalence was higher in our study); however our numbers are too low to make direct comparisons and the age groups, while similar, are not exact.

The incidence of *B. parapertussis* was lower than for *B. pertussis* although the difference was not statistically significant. Our incidence of 3.0 cases per 1000 person-years was comparable to that of 2.1 per 1000 person-years found in the Italian acellular pertussis vaccine trial in 1992-93[22,39]. The duration of illness was shorter for *B. parapertussis* with a maximum duration of 6 days compared to a maximum of 33 days for *B. pertussis*. A milder presentation is consistent with clinical knowledge of *B. parapertussis* infection[39-41]. *B. parapertussis* cases occurred in a later season (September through February) than for *B. pertussis* (February through June).

The low number of pertussis cases detected limited our ability to statistically test for differences between (1) respiratory episodes with confirmed pertussis versus all respiratory episodes and between (2) pertussis and non-pertussis

cases. While the differences were not statistically significant pertussis episodes were more likely (>10% absolute difference) to include cough, wheeze, cough with vomit, and whoop compared to all respiratory episodes [Table 3]. The average pertussis episode was longer (13 days versus 5 days) than all respiratory episodes. For pertussis cases it was observed that pertussis infants were lower birthweight, younger gestational age, and had older age at first pertussis vaccination although the differences are not confirmed with statistical testing. Further, mothers of infants who experienced pertussis were more likely to be literate, primiparous, and Pahadi ethnicity.

Limitations

There were several study design limitations. Pertussis is a cyclical disease, thought to peak every 2 to 4 years[42]. While we captured a full two years of surveillance data we may have captured the burden at a low period in its cycle.

We assumed when *B. pertussis* and *B. parapertussis* were isolated they were the causative agent of the respiratory symptoms triggering the nasal swab. However, we cannot be certain whether pertussis caused the exhibited symptoms, another organism triggered the illness or if symptoms were related to two or more etiologic agents. While co-infections with *B. pertussis* and *B. parapertussis* have been widely documented[33,43-48] there is mixed evidence for pertussis residing in the naspharynx of healthy individuals or long-term pertussis carriage[29,49-51]. Future analysis of the data (once available) will include an examina-

tion of pertussis co-infection with influenza, RSV, and potentially other respiratory viruses.

Our study was based within a randomized controlled trial of influenza vaccination in pregnancy for which sample size calculations and data collection methods were developed. Given the observed number of pertussis cases seen and the limit to our sample size we were unable to performed pre-specified statistical testing for characteristics associated with pertussis disease and pertussis cases.

Parents, rather than trained clinicians, reported the respiratory symptoms in their infants. Parents may have missed signs that otherwise would have been observed by a health care worker. However, the criteria for collection of the nasal swab were quite broad and largely did not require sophisticated clinical skills. Apnea and cyanosis however may have been difficult for parents to identify. In the first year of surveillance pertussis-specific symptoms were not included in the weekly morbidity assessment. To increased pertussis detection sensitivity additional pertussis-specific symptoms were added in year two, which included apnea, cyanosis, cough with vomit, and whoop. While the addition of these symptoms expanded our clinical description of cases, these additions did not lead to any additional nasal swabs collected, over and above those triggered by the symptoms collected over the first year of the study. No infant experienced a pertussis-specific symptom in isolation without also having one of the originally specified respiratory symptoms. These data support our assumption that we

were unlikely to have missed pertussis cases in year one with our less sensitive respiratory symptom criteria.

Nasal swabs were collected in the mid-nasal region for the primary purpose of influenza virus detection. The mid-nasal area was selected as influenza is able to be isolated from this area and this procedure is less invasive and causes less discomfort than nasopharyngeal area collection. In a field site (non-clinical setting) the acceptability of multiple nasopharyngeal swabs in a home setting would likely be limited and increase participant refusal rate. This would have decreased the generalizability of our results to the entire population. Currently, the mid-nasal region is not recommended as a specimen collection site for pertussis. Nasopharyngeal swabs or nasopharyngeal aspirates are the recommended specimen collection method[52-54]. However, the nasopharyngeal region was established as the collection area of choice when the sole diagnostic measure was culture, which has low sensitivity. No published studies to-date specifically examined the feasibility of mid-nasal swabs for pertussis using the highly sensitive PCR diagnostic method[17,55,56]. One study tested nasal swabs for pertussis with 40% testing positive (no gold standard for comparison), demonstrating the possibility of detecting pertussis in the mid-nasal region[57]. A study by our collaborators, currently under peer review, found that pertussis isolation from nasopharyngeal swabs was comparable to that from the mid-nasal region at Seattle Children's hospital from 2011-2012[58]. Our study was able to isolate pertussis DNA from the mid-nasal region. A limitation of our study is that the collection area may have lowered the sensitivity of pertussis detection. However, given the un-

published results from Washington we believe the isolation method is adequate for pertussis identification.

Strengths

Strengths of the study are that it was a population-based, prospective study, with very low refusal rates that identified risk factors and clinical symptoms without the potential bias that may occur when these data are collected retrospectively. The community-based design allows generalizability of these results to the entire population and not just those seeking care at a health facility or those in an outbreak situation. Sarlahi district, located in the terai region where the majority of Nepalese reside, has similar demographics to the entire population. Sarlahi's location near sea level and on the border with India supports the generalizability of these results to many populations living on the Indian subcontinent[59]. The weekly active surveillance with sensitive criteria for pertussis testing was able to detect mild and atypical pertussis cases, which may have been missed by previous traditional surveillance. The multi-target PCR method allowed highly sensitive and specific detection of two additional *Bordetella* species beyond the primary *B. pertussis* target.

Conclusion

An epidemic level of pertussis seen in some countries was not observed in Nepalese infants. Whole cell vaccine appears to be protective of infants in this environment given the low incidence of pertussis during the study period. Pertussis cases were generally milder than expected compared to traditional pertussis clinical definitions. These data support clinicians considering pertussis in their differential diagnosis of infants with mild respiratory symptoms. Policy-makers in Nepal will need to weigh the benefit of an additional prenatal pertussis vaccine or a switch to acellular primary pertussis vaccine with the low burden of pertussis in infants less than 6 months.

Our study demonstrated that mid-nasal swabs are able to isolate pertussis for detection by multi-target PCR. Further research is needed comparing the mid-nasal to nasopharyngeal isolation areas. The less invasive mid-nasal nasal swab is an attractive alternative for pertussis nasal swab collection. This method may in the future enhance population-based surveillance efforts, which are limited in part by the need for nasopharyngeal swabs/aspirates.

Table 4.1A - Pertussis Episode Characteristics

Case	Pertussis Start Date	Age (days)	Duration (days)	Fever	Cough	Wheeze	Difficulty Breathing	Ear Infection	Cyanosis	Apnea	Whoop	Cough with Vomitting
[A]1	9-Feb-12	63	33		+		+		NA[B]	NA	NA	NA
2	2-Mar-13	102	4	+	+	+	+				+	+
3	20-Feb-13	109	7		+	+	+					
4	7-Mar-13	97	19	+	+							
5	14-Apr-13	68	2	+		+						
6	10-Mar-13	19	10	+	+	+						
7	27-Jun-13	44	13			+					+	+

Table 4.1B - Maternal & Household Characteristics

Case	Parity	Ethnicity	SES Quartile	Age	Literacy	Years in School	Tobacco	Household Members	Children Under 5 Years	Children Under 15 Years
1	First Pregnancy	Madeshi	3	20		0		21	3	9
2	First Pregnancy	Pahdai	2	19	+	10		6	0	0
3	First Pregnancy	Pahdai	1	19	+	10		6	0	0
4	Non First Pregnancy	Pahdai	3	35	+	5		7	1	2
5	First Pregnancy	Madeshi	1	18	+	6		8	1	3
6	Non First Pregnancy	Pahdai	3	32	+	4		4	1	2
7	First Pregnancy	Pahdai	4	19	+	7		13	0	4

Table 4.1C - Infant Characteristics

Case	Birthdate	Sex	Gestational Age (weeks)	Preterm	Birthweight (grams)	Low birthweight	SGA (<10%)	SGA (<3%)	Breastfed <1 hour after birth	1 Pertussis Vaccination by 6 Months	Age at 1st Pertussis Vaccination (Days)	Pertussis Vaccination Before Illness Onset	Spacing between vaccination and ilness (Days)	Underweight <-1 z-score at 6 months	Stunting <-1 z-score at 6 months	Wasting <-1 z-score at 6 months
1	8-Dec-11	Male	40.6		2000	+	SGA<10%	SGA <3%		+	68			+		+
2	20-Nov-12	Female	35.9	+	2390	+	Normal	Normal	+	+	86	+	16			
3	3-Nov-12	Female	40.7		.[C]	.	.	.		+	104	+	5	.	.	.
4	30-Nov-12	Female	38.6		.	.	.	.		+	75	+	22	+	+	
5	5-Feb-13	Male	38.0		2520		SGA<10%	SGA <3%			.			+	+	+
6	19-Feb-13	Male	38.9		3130		Normal	Normal		+	158					
7	14-May-13	Male	35.3	+	2410	+	Normal	Normal	+		.			.	.	.

Table 4.1A-C Footnotes:

A – Infant had 2 positive nasal swabs associated with this pertussis episode; all other infants had only 1 positive nasal swab

B - Cyanosis, apnea, whoop, and cough with vomiting were asked in year 2 only so this infant does not have this data

C – Missing data

Table 4.2A - Parapertussis Episode Characteristics

Case	Pertussis Start Date	Age (days)	Duration (days)	Fever	Cough	Wheeze	Difficulty Breathing	Ear Infection
1	9-Sep-11	49	3				+	
2	5-Oct-11	67	6	+	+	+		
3	17-Feb-12	71	4		+	+		
4	24-Dec-11	7	2			+		

Table 4.2B - Maternal & Household Characteristics

Case	Parity	Ethnicity	SES Quartile[A]	Age	Literacy	Years in School	Tobacco	Household Members	Children Under 5 Years	Children Under 15 Years
1	Non First Pregnancy	Pahdai	2	27	+	10		4	2	2
2	Non First Pregnancy	Pahdai	2	26		0		6	2	2
3	Non First Pregnancy	Madeshi	3	28	+	5		11	2	3
4	First Pregnancy	Pahdai	4	21	+	6		15	1	3

Table 4.2C - Infant Characteristics

Case	Birthdate	Sex	Gestational Age (weeks)	Preterm	Birthweight (grams)	Low birthweight	SGA (<10%)	SGA (<3%)	Breastfed <1 hour after birth	1 Pertussis Vaccination by 6 Months	Age at 1st Pertussis Vaccination (Days)	Pertussis Vaccination Before Illness Onset	Spacing between vaccination and illness (Days)	Underweight <-1 z-score at 6 months	Stunting <-1 z-score at 6 months	Wasting <-1 z-score at 6 months
1	22-Jul-11	Male	36.1	+	.[B]	.	.	.	+	+	118					
2	30-Jul-11	Male	40.6		2910		SGA<10%	Normal	+	+	80					
3	8-Dec-11	Female	41.0		3310		Normal	Normal		+	101			+		+
4	17-Dec-11	Male	42.7		3640		Normal	Normal						.	.	.

Table 4.2A-C Footnotes:

A – SES - 4th = top income bracket

B – Missing data

Table 4.3 - Characteristics of All Episodes and Pertussis Episodes*

Characteristic	All episodes (N=2924)		Pertussis Episodes (N=7)	
Fever - no (%)	1,405	48%	4	57%
Cough - no (%)	1,772	61%	5	71%
Wheeze - no (%)	1,300	44%	5	71%
Difficulty breathing - no (%)	1,159	40%	3	43%
Ear Infection - no (%)	141	5%	0	0%
Cyanosis - no (%)**	7	1%	0	0%
Apnea - no (%)	57	4%	0	0%
Cough with vomit - no (%)	137	11%	2	33%
Whoop - no (%)	79	6%	2	33%
Episode start age - (days)	88 ±	48	73 ±	33
Episode duration - (days)	5 ±	4	13 ±	11
1st pertussis vaccination - no (%)	1,081	37%	3	43%
Space between vaccination and episode - (days)	48 ±	35	14 ±	9

* Plus–minus values are means ±SD.

** Year 2 has n=1,292 for all episodes and n=7 for pertussis episodes (cyanosis, apnea, cough with vomit, and whoop)

Table 4.4 - Characteristics of Subjects in the Population and Pertussis Cases*

Characteristic	Population** (N=3235)	Pertussis Cases (N=7)
Male Sex - no (%)	1,708 53%	3 43%
Gestational Age - weeks	39.5 ± 3.7	38.6 ± 2.1
Birthweight - (g)	2791 ± 496	2502 ± 408
Small-for-Gestational Age - no (%)	694 27%	2 40%
Age at 1st Pertussis Vaccine - days	83 ± 35	98 ± 36
6 Month Underweight - (z-score)	-0.78 ± 3.66	-1.37 ± 1.38
6 Month Stunting - (z-score)	-0.87 ± 1.39	-0.85 ± 0.83
6 Month Wasting - (z-score)	-0.34 ± 2.76	-1.13 ± 1.45
Mom Age - years	23.5 ± 4.7	23.1 ± 7.2
Breastfeeding in 1st Hour - no (%)	1,106 35%	2 40%
Literate - no (%)	1,730 60%	6 86%
Primiparous - no (%)	1,186 41%	5 71%
Pahadi Ethnicity - no (%)	1,697 58%	5 71%
Highest quaritile SES - no (%)	680 23%	1 14%
Household Number	8.6 ± 4.2	9.3 ± 5.9
Children under 15 years	2.5 ± 2.1	0.9 ± 1.1
Children under 5 years	1.2 ± 1.3	2.9 ± 3.1

* Plus–minus values are means ±SD.

** Pertussis Cases are included in total population

Chapter 4 References

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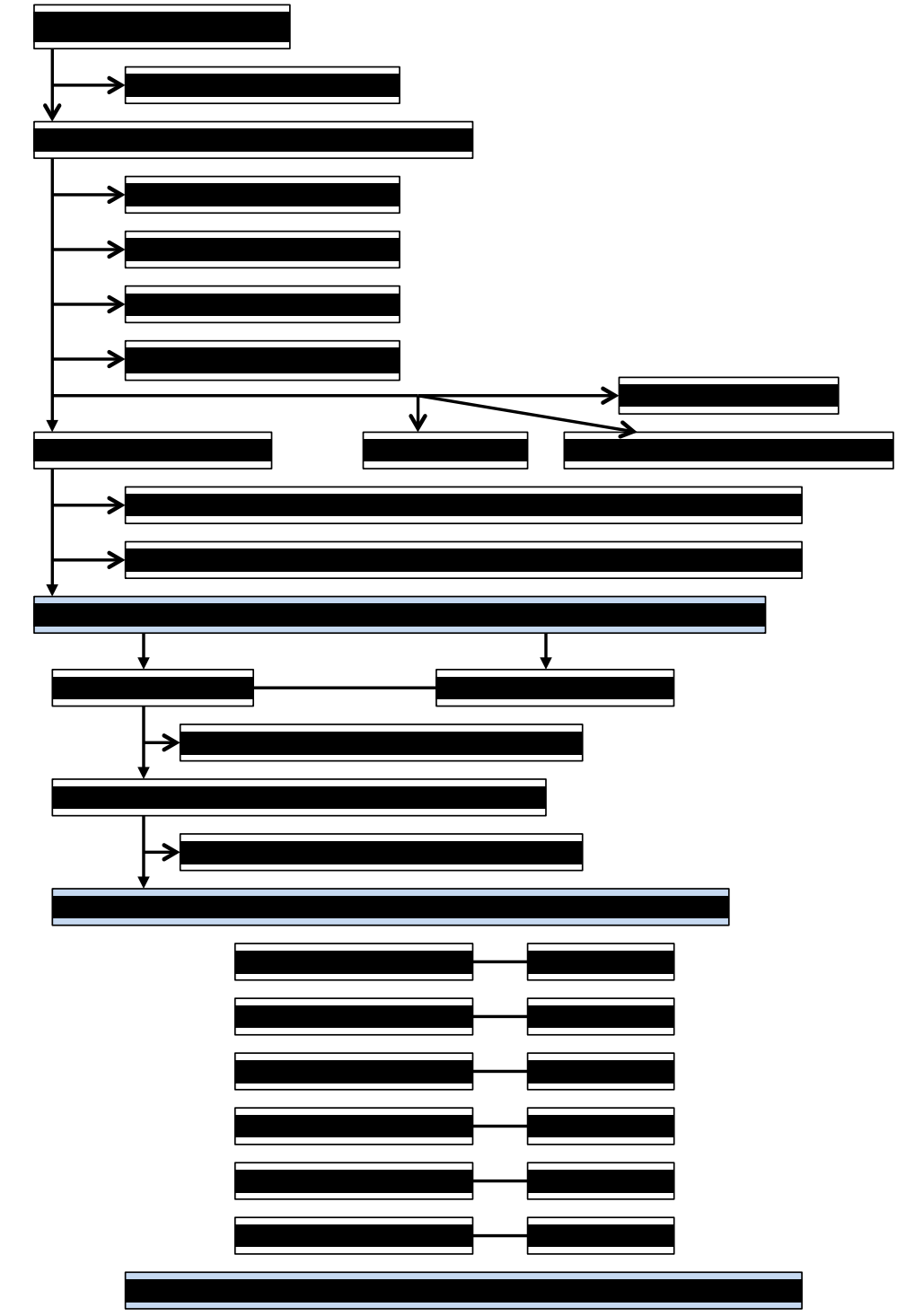
Chapter 4 Appendices

Appendix 4.1

PCR primer targets and Melting Temperatures					
Primer Type and Name	Sequence	Amplicon Length (bp)	*B. pertussis*	*B. parapertussis*	*B. bronchiseptica*
			Melting Temperatures		
IS*481* (IS)		182	85-86°C	-	-
IS-F	GATTCAATAGGTTGTATGCATGGTTC				
IS-R	TTCAGGCACACAAACTTGATGGGCG				
ptx promoter (PT)		189	87-88°C	89-89.5°C	90°C
PT-F1	CCAACGCGCATGCGTGCAGATTCG				
PT-F2	CCAACGCGTATGCGTGCGGATGCG				
PT-R1	CTCTGCGTTTTGATGGTGCCTATT				
PT-R2	CTCTGCGTTTCGGTGGTGCCTATT				

Appendix 4.2

Population Selection Chart



CHAPTER 5

Pertussis Toxin Antibody Transfer in Mothers and Infants in Sarlahi, Nepal

Authors

Michelle Hughes, Janet Englund, James Tielsch, Michael Rock, Kathryn Edwards, Mark Steinhoff, Steve LeClerq, Subarna Khatry and Joanne Katz.

Abstract

Background: Pertussis is estimated to cause 2% of childhood deaths globally and is a growing public health problem with infants at greatest risk of morbidity and mortality. Maternal vaccination during pregnancy may be effective to prevent pertussis in young infants but an understanding of pertussis antibody levels and the efficacy of maternal to infant antibody transfer in a low-income South Asian setting is lacking.

Objective: To estimate the level of pertussis toxin antibody and the efficiency of its transfer from mothers to infant in Sarlahi District, Nepal.

Design/Methods: Nested within a randomized controlled trial of influenza vaccination during pregnancy, a subset of paired mothers and infants' blood samples were collected at delivery. Serum was tested for pertussis toxin (PT) antibodies using an ELISA. PT antibody levels and the maternal to infant transfer efficiency were estimated.

Results: The PT infant to mother ratio was 1.1 (95% CI: 1.0 – 1.2) for 131 mother-infant pairs. Mother and infant pairs with detectable PT antibody were correlated but the majority of mothers and infants had antibody levels below the level of quantification

Conclusions: Maternal and infant PT antibody levels were low in rural Nepal. While overall transport was active and there was an association between mother and infant PT antibody levels, a large proportion of infants had antibody levels below their mother's level. Maternal immunization could be an important intervention to support infant pertussis immunity before infants are fully vaccinated.

Introduction

Epidemic levels of pertussis have been reported in several countries in recent years[1-8]. Age groups particularly affected include infants and adolescents[9,10]. The resurgence of infant pertussis is of greatest concern as infants are at highest risk for severe morbidity and mortality compared to other age groups[3,11,12].

Several strategies are available to protect infants in countries where booster acellular pertussis vaccines widely used. Adolescent vaccination provides immune boosting to reduce the number of susceptible persons who might transmit pertussis to infants although its effectiveness in protecting infants is unclear[13]. Parents and close caregivers are most likely to infect infants[14]; vaccinating these close contacts as a cocooning strategy has also been implemented. High vaccination costs, feasibility of vaccinating all contacts, and lack of demonstrated efficacy continue to hamper efforts[3]. The most promising strategy to protect infants is vaccination of women during pregnancy so they are able to passively transmit pertussis antibodies through the placenta during gestation and breastmilk after birth[15,16]. Randomized control trials of pertussis vaccine in pregnancy were recently conducted in the United States[17] and Canada[18].

While several studies have examined the level of pertussis antibodies in mothers and infants and the efficacy of transfer[19-27], none of these has been conducted in a whole-cell vaccine using low-income country where malnutrition and prematurity are high. The goal of our study was to quantify maternal and infant pertussis toxin (PT) antibody and the transfer efficacy in a south Asian set-

ting. Maternal immunization may be a promising strategy to protect infants in this setting but a better understanding of population-level maternal antibody, the efficacy of transfer to infants, and factors which modify these levels is needed to inform prevention strategies.

Methods

Settings and population

The setting of the study was Sarlahi District, located in the central terai (low lying plains) region of Nepal. The study was nested within a randomized controlled trial of maternal influenza vaccination during pregnancy. At the start of the trial, prevalent pregnancies were identified through a survey census of all households in the catchment area. The head of the household was read an informed consent script and asked for consent to participate in the trial. For the duration of the trial field workers visited all households in the community where married women (15 – 40 years) resided every 5 weeks for surveillance of incident pregnancies. Once a pregnancy was identified women were asked for their individual consent to participate in the trial. Over a two-year period between April 25, 2011 and April 24, 2013 women between 17-34 weeks gestation were enrolled and randomized to receive either an influenza vaccine or placebo. Due to randomization of gestational age at vaccination, the last participant enrolled in the trial received their vaccine allocation September 9, 2013. All participants received ancillary benefits, which included a 90-day supply of iron-folic acid tablets, deworming medicine (single dose of albendazole), clean birthing kit, chlorhexidine ointment for umbilical cord care, a tetanus toxoid vaccine, if indicated, and health education messages, in addition to antenatal services according to the local standard of care. The study was a prospective cohort of mother-infants pairs. Approval for the study was obtained from the Johns Hopkins Bloomberg School

of Public Health Institutional Review Board and the local ethical review board (Institute of Medicine at Tribhuvan University/Nepal Health Research Council).

Data Collection

At baseline, information was collected on household structure, socioeconomic status, and demographics. At study enrollment, date of last menstrual period and pregnancy history data were collected. A sterile plastic container was left with the mother prior to delivery. Mothers were requested to collect at least 2-5cc of umbilical cord blood as soon as the placenta was delivered. The mothers then notified the local study team member of the birth so a trained worker could visit the house to collect the birth information, cord blood, and infant weight. For mothers who delivered in health facilities in the area, facility staff obtained cord blood, which was then collected by study staff for normal processing. The blood was transported on ice to the central field-processing laboratory where it was centrifuged after sufficient clotting. Sera was removed, aliquoted into cryovials and placed in liquid nitrogen. Maternal blood was collected from approximately 1 week post-partum. Venous blood (~5cc) was collected from mothers' arms and processed using the same technique as the infant cord blood. Serum samples were stored and shipped to the United States at -80° Celsius.

Laboratory Assays

The immunoglobulin G (IgG) anti-PT enzyme-linked immunosorbent assay (ELISA) was performed at Vanderbilt University School of Medicine according to previously described methods[28]. The reference standard was pertussis antiserum

(human), lot 3 (CBER3 [US Food and Drug Administration]). The lower level of quantification (LOQ) was 10 ELISA(EU)/mL.

Analytic Dataset

Mother-infants pairs were included in this analysis if they both had a delivery time point blood sample collected. See Appendix 5.1 – Population Selection for detailed sample selection procedures. Of 1,426 unique maternal and infant blood samples at delivery there were 908 maternal delivery and 518 infant cord blood samples. 637 mothers and 464 infant samples were matched to records of maternal vaccination and infant birth assessment. For 487 of these samples only the mother or infant were available (non-paired). The resulting 614 paired samples (307 pairs) had testing completed for 262 samples (131 pairs). Complete data from the trial including testing of the remaining 352 samples are pending.

At baseline, data on household structure was gathered, including age and sex of all household members. Binary variables for household density were created for all household members (>10 persons), those less than 15 years (>3 children under 15 years), and those less than 5 years (>1 child under 5 years). At enrollment women reported their literacy status (binary) and pregnancy history. The field workers identified their ethnicity (Pahadi or Madeshi) from names and observation. For parity analysis women were categorized as nulliparous or multiparous. Responses to twenty-five questions were used to develop a construct to measure the socioeconomic status (SES) of households. The results were averaged and divided into SES quartiles for analysis.

Gestational age was measured using a woman's report of date of last menstrual period during pregnancy surveillance (an average of 3-4 weeks recall). Gestational ages <37 complete weeks were categorized as preterm. Birthweight was collected at the home as soon as possible after birth using a digital scale [Tanita model BD-585, precision to nearest 10g]. Birthweights collected >72 hours after birth were excluded from the analysis of birthweight. Infants were categorized as low birthweight if weight was <2500 grams. Small for gestational age (SGA) was calculated using the sex-specific 10th percentile cut-off described by Alexander[29].

Statistical Analysis

Individual Mother and Infant Antibody Titers

PT Antibody levels below the LOQ were assigned one-half of the assay LOQ (5 EU/mL). Geometric mean concentrations (GMC) and bootstrapped-derived 95% confidence intervals were constructed separately for mothers and infants. Reverse cumulative distribution curves were created to visualize and compare the distribution of log transformed antibody titers for mothers and infants.[30] To examine differences in PT levels by infant, maternal, and household characteristics, non-parametric testing was performed for binary (Wilcoxon rank sum test with continuity correction) and nominal (Kruskal-Wallis rank sum test) variables. For this testing, continuous predictors were transformed to dichotomous and nominal factors. Bivariable and multivariable logistic regression models were used to assess the association of risk factors with the presence of PT

antibodies separately for mothers and infants. Antibody was modeled as a binary outcome as the distribution remained non-normal after log transformation.

Infant to Mother Antibody Ratio

The ratio of infant to maternal PT GMC was calculated both for all mother-infant pairs and for the subset of mother infant pairs where at least one of the pair had an antibody level above the LOQ. This subset therefore excluded pairs where both mother and infant had antibody level below the LOQ. Additional analysis of the transfer ratio was restricted to the pairs in this subset.

Spearman's rank correlation rho was calculated for the correlation of mother and infant PT antibody. Unadjusted and adjusted linear regression models were developed for examining the association of log ratio of infant to maternal PT antibody levels with infant, maternal, and household characteristics.

The cutoff for statistical significance in all testing was $p<0.05$. All statistical analyses were conducted in R version 3.0.2 (2013-09-25).

Results

Maternal and Infant Antibodies

Maternal post-partum and infant cord blood were collected from 131 mother-infant pairs between May 12, 2012 and August 12, 2013. The range of maternal blood collection was 5-141 days post-partum; infant collection ranged from day of birth to 6 days post-birth. The majority (71%) of women delivered in a health facility. Infant PT GMC was 11.5 (95% CI: 9.8 – 13.5) EU/mL [Range 5 – 258]. Maternal PT GMC was 10.6 (95% CI: 9.0 – 12.4) EU/mL [Range 5-145]. The majority of mothers and infants had PT antibody below the lower level of quantification (LOQ), 10 EU/mL. Forty-three percent of infants and 40% of mothers had antibody above our LOQ [Figure 5.1]. Five mothers (3.8%) in our sample had PT IgG >100 EU/mL, indicative of recent pertussis infection.

Mothers

Mothers who were illiterate and of Madeshi ethnicity had statistically significantly higher PT antibody than mothers who were literate or Pahadi ethnicity [Table 5.1].

Illiteracy and Madeshi ethnicity were the only two characteristics associated with presence of maternal PT antibody in bivariate logistic regression models [Table 5.2]. In a multivariate model adjusting for all potential predictors, literacy remained statistically significant with literate mothers having a 75% (95% CI: 32-91%) decreased odds of having detectable PT antibody compared to mothers who were illiterate. Infant gestational age was also associated in the multivariate

model. For every week increased infant gestational age, mothers had 27% (95% CI: 2 – 62%) increased odds of having detectable PT antibody.

Infants

No statistically significant differences in infant PT antibody levels were found by infant, maternal, or household characteristics [Table 5.1].

In a bivariate logistic regression model for presence of PT antibody, gestational age was the only significant predictor of antibody presence in infants [Table 5.3]. Infants born at one week greater gestational age had 23% (95% CI: 5-47%) increased odds of having detectable PT antibody compared to infants born a week prior. In an adjusted model, while the gestational age odds ratio estimate remained the same it was no longer statistically significant.

Association of Maternal and Infant Antibodies

The placental transfer estimate was 1.1 (95% CI: 1.0 – 1.2), indicating active antibody transport from mothers to their infants [Table 5.4]. A total of 62 mother-infant pairs had at least one of the pairing (mother or infant) with antibody above the LOQ. In this subset of 62 pairs, placental transfer was 1.2 (95% CI: 1.1 – 1.4). Active transfer, equal transfer, and negative transfer were found for 62%, 2%, and 35% of pairs respectively. Maternal and infant antibody levels were highly correlated (Spearman's rank correlation, 0.82 ($p<0.0001$)), excluding pairs where both had non-detectable PT antibodies) [Figure 5.2].

Figure 3 shows the relationship between antibody transfer and maternal, infant, and household characteristics. In an unadjusted linear regression model of the

log-transformed transfer ratio, maternal literacy and higher gestational age were the only variables associated with statistically significantly higher antibody transport [Table 5.5]. For each week of increasing gestational age the ratio of infant to maternal antibody had an absolute increase of 8% (95% CI: 0-16%). In the multivariable model literacy remained statistically significant with transfer for literate mothers 57% (95%: 2-139%) higher than for illiterate mothers. Similar to the multivariable logistic model for presence of PT antibody in mothers, gestational age had a similar estimate compared to the bivariate model but did not reach statistical significance.

Discussion

Antibody transfer was low with an infant to maternal PT IgG ratio of 110%. While the majority of pairs with detectable level of PT antibody had active transport from mother to infant, a sizeable minority of infants (35%) had lower PT antibody than their mothers. IgG is transferred from mother to fetus through the placenta beginning at approximately 16 weeks gestation, increasing until delivery[20,31,32]. Studies from the 1940s found low efficiency of maternal to infant PT IgG transport with only 2-12% of newborns having higher antibody levels than their mothers[31]. Mothers who previously contracted pertussis or were immunized with whole cell pertussis vaccine during pregnancy gave birth to infants with the highest pertussis antibody titers. Recent studies have found a strong association between mothers and infants with active PT antibody transport; PT antibody transfer ranged from 107-169% (excludes preterm infants in studies where only separate transfer ratios reported)[19-27]. While we found high correlation between mothers and infants, the transfer in our study population was on the lower end of expected values. Studies rarely publish the proportion of pairs with negative transport so we are unable to compare whether the 35% we observed is comparable to other populations.

Older gestational age and maternal literacy were associated with higher transport compared to infants born at younger gestational age and to illiterate mothers. The association of preterm birth with lower transport is consistent with previous research in a variety of populations[21,25,33]. A biological explanation for lower transport in preterm infants is that IgG transfer increases during gestation

leaving infants born earlier with less opportunity for maternal transfer[25]. Maternal literacy was associated with increased transport and remained the only significant predictor even when controlling for several infant, maternal, and household characteristics. Maternal literacy likely has no biological impact on PT IgG transfer but serves as a proxy for an unmeasured variable(s) directly influencing placental transfer. Other factors which have been associated with transport are maternal ethnicity, pertussis vaccination, HIV and health status[23,24,33,34]. We found no difference in transport by sex, birthweight, parity, ethnicity, number of household members or socioeconomic status.

Sixty percent of mothers had PT antibody level below our level of quantification. While not directly comparable, pertussis antibodies were detected in 30-50% of women in the pre-vaccine era[35,36], while a lower percentage of contemporary women harbor high levels of pertussis antibodies[19,37-39]. A recent study in the U.S. found comparable levels to ours with only a fifth of women at delivery with PT antibody levels >5 EU/mL[27]. In Nepal, maternal antibodies are either due to lingering immunity from the childhood vaccination series or a previous pertussis infection. As there are no adolescent or adult boosters in Nepal high PT antibodies in mothers are likely due to recent infection. Approximately 4% of mothers had antibody levels, which suggest a recent pertussis infection. While there is no serological correlate of immunity, PT IgG antibody titers above 100 EU/mL are considered indicative of recent infection[40,41].

Women of Madeshi ethnicity or those who were illiterate were more likely to have detectable and higher levels of PT antibody than mothers who were Pa-

hadi or literate. In our adjusted model literacy remained statistically significant and increased infant gestational age was associated with higher maternal antibodies. One explanation could be that the Madehsi and illiterate population have lower health status and therefore are more susceptible to pertussis infection. By contracting pertussis themselves, these mothers have higher antibodies than mothers who remained pertussis uninfected. While infant gestational age cannot causally link to maternal PT levels we found an association of older gestational age with increased odds of maternal PT antibody. Potential unmeasured confounders might affect both gestational age and maternal antibody thus resulting in the association observed. The literature on risk factors for maternal PT antibody levels is sparse. Mothers with a chronic disease(s) were found to have lower PT antibodies than mothers with no chronic health condition[39]. However, HIV infection was not shown to be associated with lower PT antibodies compared to those mothers who are HIV negative[23].

Over half of infants had PT antibody levels below our level of quantification. Infant PT antibody at birth is the result of maternal placental transfer during gestation and is dependent on the maternal level. A recent study found only a quarter of infants at birth had PT antibody > 5 EU[27]. Passively derived PT antibodies in infants can protect newborns from pertussis until they are able to be vaccinated themselves[42]. The low level seen likely indicates that the majority infants are susceptible to pertussis from birth.

No characteristics were associated with the level of PT antibody in infants. In a model for the presence of PT antibodies gestational age was the only factor

associated with increased odds for detectable PT antibody. Factors previously shown to decrease infant PT antibody titers were lower maternal age, maternal HIV infection, and being born preterm[21,23,25,27,33]. Infants whose mothers have higher antibody levels due to recent infection or vaccination have higher antibody levels than infants of unexposed or unvaccinated mothers[24,27,43]. Pre-term infants have lower antibody concentrations compared to term infants[21,25,33]. Pregnancy history, occupation, education, ethnicity, marital status and number of household members have previously been found to have no association with infant PT antibody levels[27]. The effect of maternal age on infant antibody levels is mixed with some studies showing an association with increased maternal age and higher PT antibody[23,27]. Our results showing gestational age is the primary factor for the presence of PT antibodies are consistent with these prior studies.

One limitation of our study is that we only measured antibodies to PT and did not measure antibodies to other antigens thought to be important in pertussis infection including filamentous hemagglutinin (FHA), pertactin (Prn), fimbriae (FIM) type 2 and type 3. With limited resources, PT was chosen as the primary antigen of choice given its specificity to pertussis in comparison to other candidates, which can be stimulated by other similar pathogens and its central importance to pertussis pathogenesis[44] Recently available funding will allow future analysis of these samples for FHA, Prn, and FIM.

Another limitation is that while our target population was population-based, the study is a convenience sample of those for which we collected paired mother and infant blood. Our results may not be representative of the entire community if

the samples are from a skewed distribution of the population, which was more likely to have blood collected.

Our sample size was limited in this initial analysis. When complete trial data are available we will have more than twice the sample size presented here. The power of our study to detect associations with PT antibody and transfer will be increased.

A third limitation is that we cannot assess whether the antibody levels observed are associated with protection from pertussis. While some classification levels have been described for PT IgG for protection and recent infection, there is no universal level for comparison of our results to those of other studies[40]

Strengths of this study are that it was a large study of paired infant and maternal pertussis antibody levels in a setting of whole cell pertussis vaccination. This was the first study, to our knowledge, to be conducted in a rural South Asian setting. Further it captured several infant, maternal and household factors not typically analyzed in combination with pertussis antibody levels and transfer.

Conclusion

Low PT antibody levels were found in mothers and their newborn infants in Sarlahi District, Nepal. When PT antibodies were present, the majority of transfer was active between mothers and their infants. Despite high correlation between mothers and infants a substantial proportion of infants had lower levels than their mothers. If the infant disease burden in Nepal justifies increased interventions, maternal immunization could be an important tool to boost maternal antibody levels to protect infants before they can be vaccinated themselves.

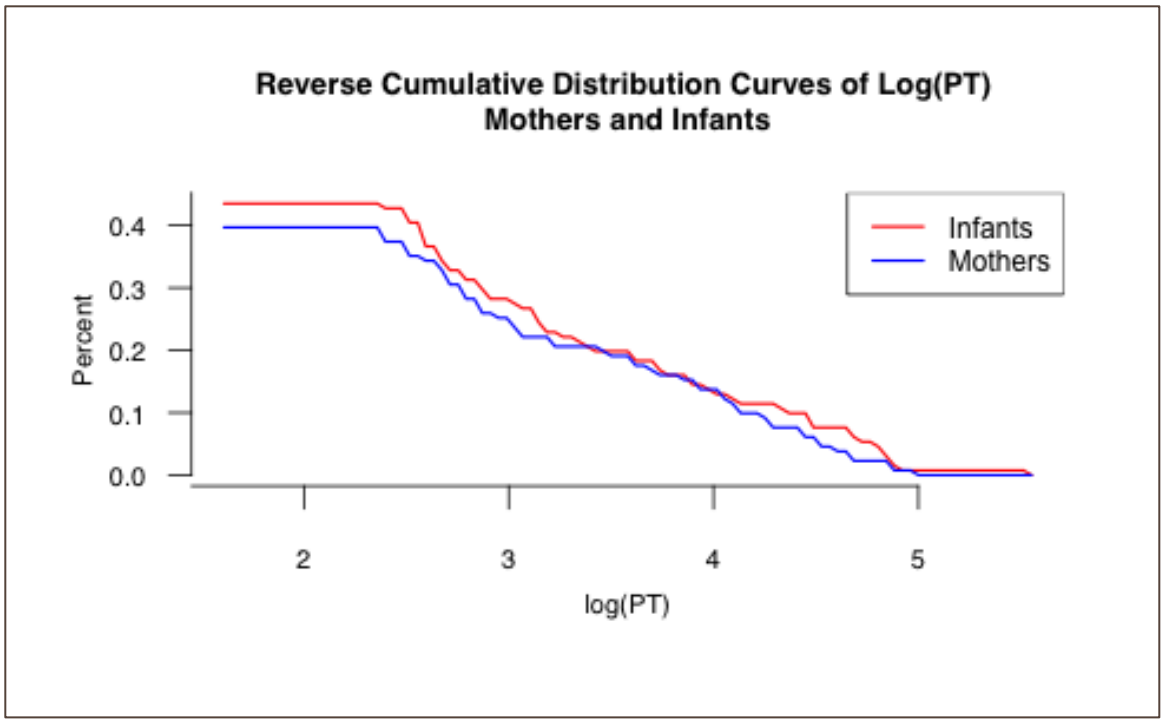

Reverse Cumulative Distribution Curves of Log(PT)
Mothers and Infants
Infants
Mothers
Percent
0.4
0.3
0.2
0.1
0.0
2
3
4
5
log(PT)

FIGURE 5.1

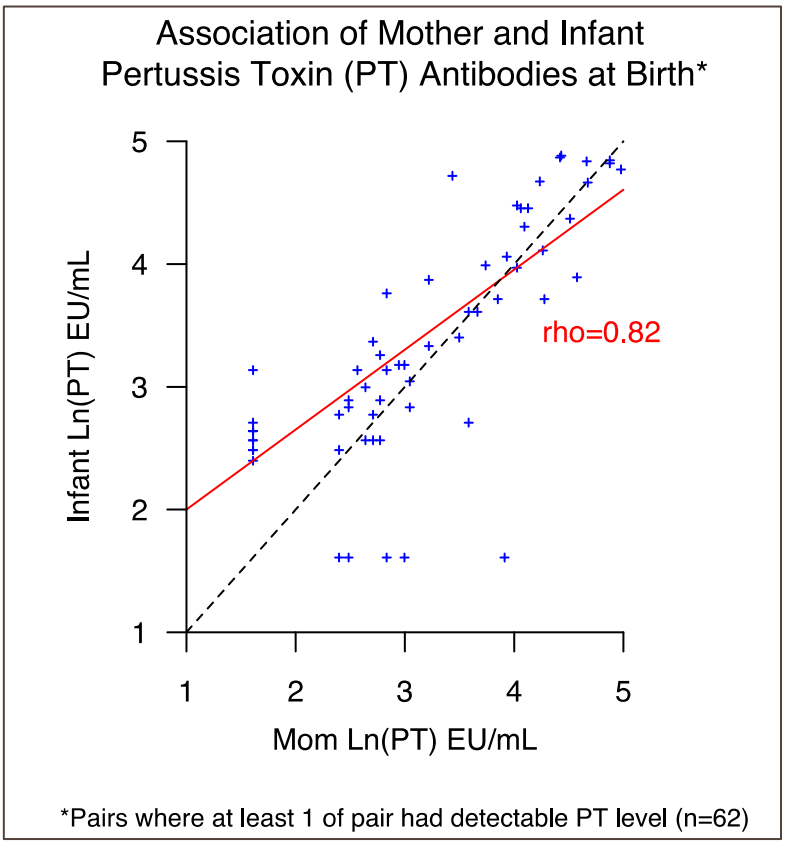

Association of Mother and Infant
Pertussis Toxin (PT) Antibodies at Birth*
Infant Ln(PT) EU/mL
Mom Ln(PT) EU/mL
rho=0.82
1
2
3
4
5
*Pairs where at least 1 of pair had detectable PT level (n=62)

FIGURE 5.2

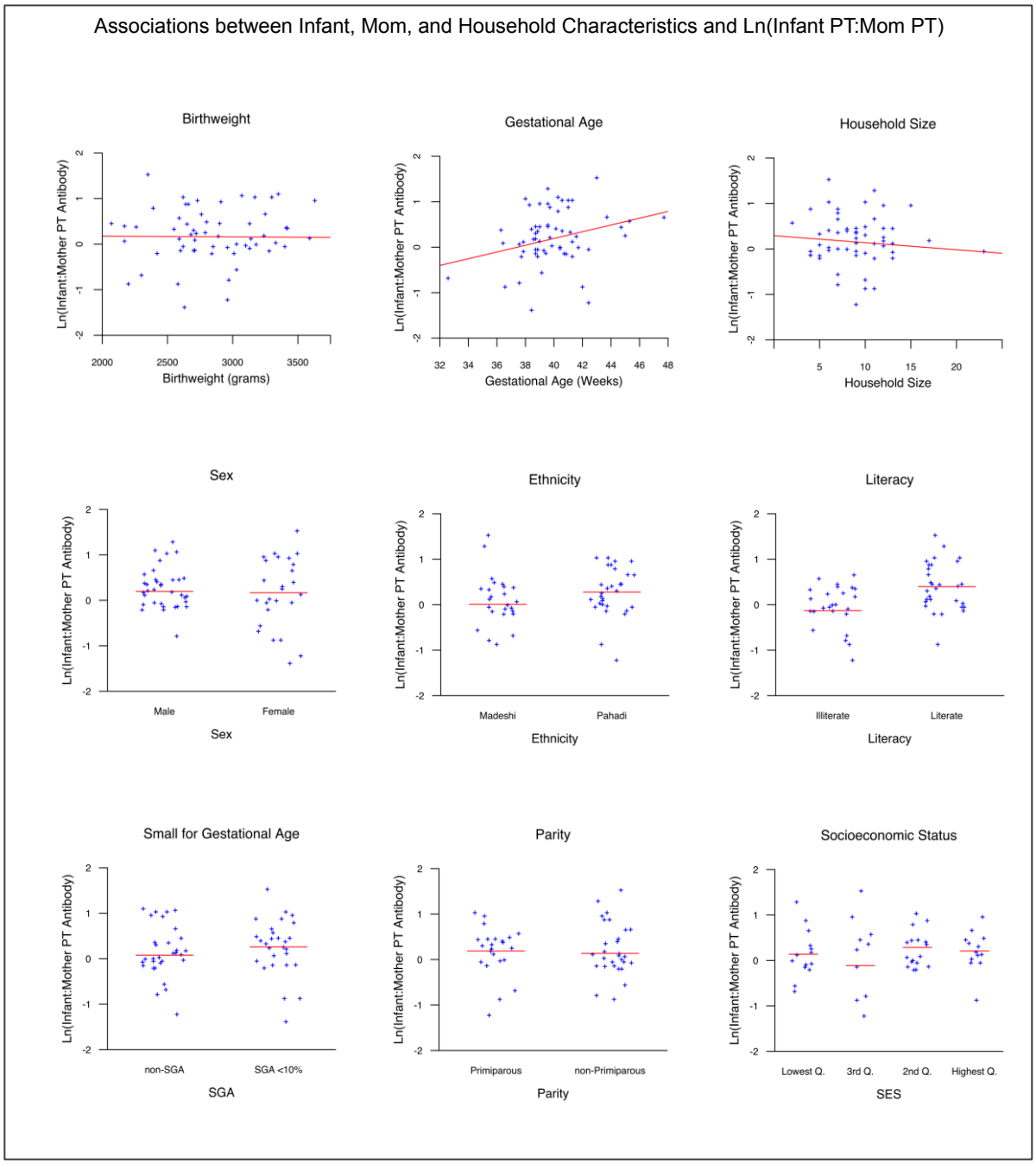

Associations between Infant, Mom, and Household Characteristics and Ln(Infant PT:Mom PT)
Birthweight
Ln(Infant:Mother PT Antibody)
Birthweight (grams)
Gestational Age
Gestational Age (Weeks)
Household Size
Household Size
Sex
Male
Female
Sex
Ethnicity
Madeshi
Pahadi
Ethnicity
Literacy
Illiterate
Literate
Literacy
Small for Gestational Age
non-SGA
SGA <10%
SGA
Parity
Primiparous
non-Primiparous
Parity
Socioeconomic Status
Lowest Q.
3rd Q.
2nd Q.
Highest Q.
SES

FIGURE 5.3

TABLE 5.1

Differences in PT Antibody by Groups

	No	(%)	Infants GMC	p-value*	Mothers GMC	p-value
Sex				0.29		0.85
Male	71	54%	12.53		11.34	
Female	60	46%	10.44		9.71	
Preterm				0.13		0.28
Term	117	89%	12.16		10.93	
Preterm	14	11%	7.36		7.96	
Low birthweight				0.56		0.39
Normal birthweight	105	81%	11.57		10.74	
Low Birthweight	24	19%	10.11		9.36	
Small for Gestational Age				0.83		0.76
AGA	70	54%	11.11		10.72	
SGA <10%	59	46%	11.50		10.17	
Literacy				0.09		**<0.01**
Literate	74	63%	10.03		8.54	
Non-literate	44	37%	14.55		15.72	
Parity				0.52		0.52
Primiparous	47	41%	12.39		11.29	
Non-primiparous	68	59%	10.66		10.04	
Ethnicity				0.06		**0.01**
Pahadi	74	63%	9.87		8.82	
Madhesi	44	37%	14.95		14.88	
SES				**0.07		0.22
Lowest Quartile	25	21%	16.35		15.16	
3rd Q.	28	24%	7.76		8.11	
2nd Q.	33	28%	14.25		12.20	
Highest Q.	32	27%	9.95		9.15	
Crowding - All				0.41		0.31
Crowded	45	38%	12.53		11.96	
Uncrowded	73	62%	10.94		10.03	
Children <5				0.38		0.25
Crowded	30	25%	12.57		12.11	
Uncrowded	88	75%	11.19		10.29	
Children <15						
Crowded	26	22%				
Uncrowded	92	78%				

*Wilcoxon rank sum test with continuity correction

**Kruskal-Wallis rank sum test

TABLE 5.2

Logistic Regression for Presence of PT Antibody in Mothers

	Unadjusted			Adjusted*		
	OR	95% CI	p-value	OR	95% CI	p-value
Female Sex	0.53	0.26 - 1.08	0.09	0.69	0.28 - 1.68	0.42
Gestational Age (weeks)	1.15	0.99 - 1.36	0.09	1.27	1.02 - 1.62	**0.04**
Birthweight (Kg)	1.34	0.59 - 3.13	0.49	0.87	0.18 - 4.29	0.87
SGA <10%	0.89	0.44 - 1.82	0.75	0.58	0.14 - 2.27	0.43
Literate	0.29	0.13 - 0.63	**<0.01**	0.25	0.09 - 0.68	**0.01**
Non-Primiparous	0.78	0.37 - 1.68	0.53	0.65	0.26 - 1.59	0.35
Pahadi Ethnicity	0.34	0.16 - 0.74	**<0.01**	0.49	0.19 - 1.27	0.14
Household number	1.11	1.00 - 1.23	0.05	1.06	0.93 - 1.21	0.36
SES**	0.88	0.63 - 1.23	0.47	1.14	0.70 - 1.87	0.60

*Full model inclusive of all variables: sex, gestational age, birthweight, SGA status, literacy status, primiparous status, ethnicity, household number, and SES

** Higher versus lower quartile

TABLE 5.3

Logistic Regression for Presence of PT Antibody in Infants

	Unadjusted			Adjusted*		
	OR	95% CI	p-value	OR	95% CI	p-value
Female Sex	0.68	0.33 - 1.36	0.27	1.03	0.45 - 2.37	0.94
Gestational Age (weeks)	1.23	1.05 - 1.47	**0.02**	1.23	1.00 - 1.55	0.06
Birthweight (Kg)	1.36	0.61 - 3.15	0.45	1.21	0.28 - 5.39	0.80
SGA <10%	1.11	0.55 - 2.25	0.76	1.14	0.33 - 4.01	0.84
Literate	0.59	0.27 - 1.25	0.17	0.59	0.23 - 1.52	0.27
Non-Primiparous	0.87	0.41 - 1.84	0.71	0.82	0.36 - 1.88	0.64
Pahadi Ethnicity	0.59	0.27 - 1.25	0.17	0.78	0.32 - 1.92	0.58
Household number	1.09	0.99 - 1.21	0.09	1.08	0.96 - 1.22	0.20
SES**	0.90	0.64 - 1.25	0.53	0.93	0.59 - 1.44	0.73

*Full model inclusive of all variables: sex, gestational age, birthweight, SGA status, literacy status, primiparous status, ethnicity, household number, and SES
** Higher versus lower quartile

TABLE 5.4

Infant to Mother Antibody Ratio

	All (n=131)		Detectable Only* (n=62)	
	GMC	**95% CI**	**GMC**	**95% CI**
Infant	11.53	9.78 - 13.50	29.20	23.71 - 35.83
Mother	10.56	9.03 - 12.36	24.29	19.68 - 29.83
Transfer Ratio	1.09	1.02 - 1.16	1.20	1.05 - 1.39

*Pairs where either the mother, infant or both had PT antibody levels above the lower level of quantification; excludes pairs where mother and infant were both below the lower level of quantification

Linear Regression for Ln(PT Infant:Mother Antibody)

Pair Characteristic	No / (%) / Mean (sd)	GMC	Unadjusted e^{β}	Unadjusted 95% CI	Unadjusted p-value	Adjusted* e^{β}	Adjusted* 95% CI	Adjusted* p-value
Sex								
Male	36 58%	1.22	1 [reference]			1 [reference]		
Female	26 42%	1.18	0.97	0.69 - 1.38	0.87	0.98	0.68 - 1.42	0.92
Gestational Age (weeks)	39.9 2.4		**1.08	1.00 - 1.16	**0.04**	1.07	0.98 - 1.16	0.14
Birthweight (Kg)	2.9 0.38		0.98	0.62 - 1.57	0.95	0.93	0.42 - 2.07	0.86
SGA <10%								
AGA	32 53%	1.08	1 [reference]			1 [reference]		
SGA <10%	28 47%	1.30	1.20	0.85 - 1.69	0.30	1.14	0.61 - 2.12	0.67
Literate								
Non-literate	26 46%	1.07	1 [reference]			1 [reference]		
Literate	30 54%	1.34	***1.70	1.24 - 2.32	**0.001**	1.57	1.02 - 2.39	**0.04**
Non-Primiparous								
Primiparous	23 43%	1.21	1 [reference]			1 [reference]		
Non-primiparous	30 57%	1.15	0.95	0.66 - 1.37	0.77	1.02	0.72 - 1.47	0.89
Pahadi Ethnicity								
Madhesi	30 54%	1.01	1 [reference]			1 [reference]		
Pahadi	26 46%	1.32	1.31	0.94 - 1.83	0.12	1.09	0.72 - 1.65	0.68
Household number	9.1 3.6		0.98	0.94 - 1.03	0.52	0.99	0.95 - 1.04	0.81
SES****	2.5 1.1		1.06	0.91 - 1.24	0.44	1.01	0.84 - 1.23	0.90

*Full model inclusive of all variables: sex, gestational age, birthweight, SGA status, literacy status, primiparous status, ethnicity, household number, and SES

**Interpretation: For every week increase in gestational age infant to maternal antibody ratio is increased by 8%

***Interpretation: Infant to maternal antibody ratio is increased 70% for pairs where the mother is literate versus pairs where the mother is non-literate

**** Higher versus lower quartile

Chapter 5 References

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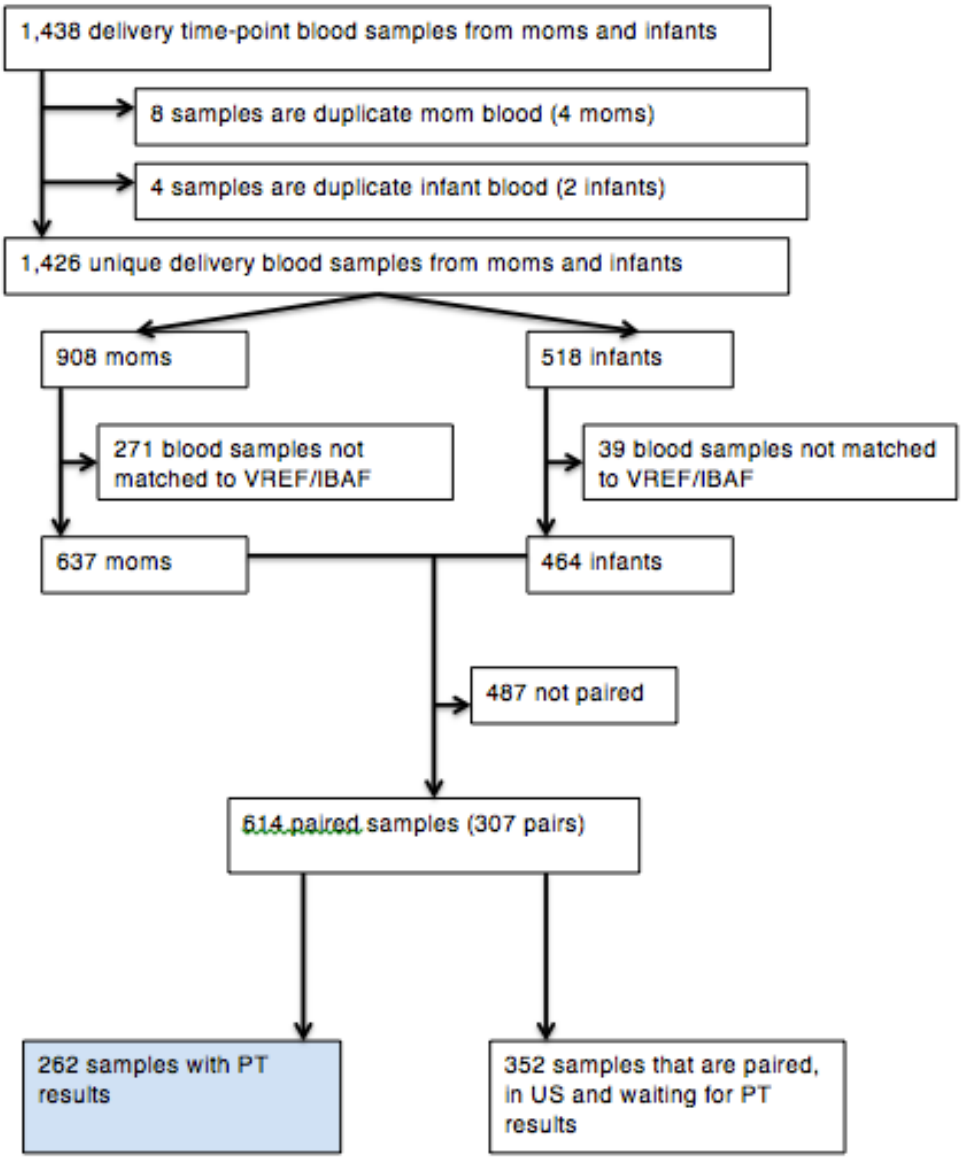
Chapter 5 Appendices

Appendix 5.1

Population Selection



CHAPTER 6

Discussion and Conclusion

Author

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Chapter 6

Discussion

The original impetus for this research stemmed from estimates that the burden of pertussis disease in children was the highest in South Asia coupled with the existence of a pertussis vaccine that would theoretically be safe and effective in pregnancy for protecting infants from pertussis[1,2]. However, key pieces of information were missing. First, there were no population-based estimates of pertussis disease burden in South Asia. Second, only one Senegal-based study had followed infants from birth to actively monitor pertussis disease in early infancy[3]. Third, the sensitivity of surveillance in prior studies was not high, potentially resulting in unawareness of milder pertussis disease. Lastly, while serological studies had been conducted in mothers and infants, most of these were conducted in high and middle-income countries, whose environments are vastly different from much of South Asia where prevalence of prematurity and malnutrition are high. The purpose of the research was to understand the epidemiology of pertussis in early infancy in Nepal to inform whether the disease burden warranted increased interventions, namely maternal pertussis vaccination.

The pertussis study harnessed the extensive resources of a maternal influenza vaccination trial and combined this infrastructure with a grant for pertussis-specific research[4]. The innovative design allowed us to add to pertussis knowledge in several ways. First, this study applied one of the most sensitive, if not the most sensitive, surveillance published for capturing infant pertussis. Study workers, who lived in the local communities, visited each household in the study every week and collected a nasal swab if any respiratory symptoms were

observed in the infant in the past week. This is in contrast to previous studies, which have generally relied on phone follow-up or parental report, had a longer duration between follow-ups, and whose criteria for testing required a minimum duration of cough (exception is the Senegal study). The greater length from commencement of symptoms to testing decreases the sensitivity of laboratory testing[5,6]. The high frequency of visits coupled with the sensitive definition allowed us to detect pertussis cases that would have been missed using traditional pertussis surveillance. Second, this is the only study of PCR-confirmed pertussis to our knowledge conducted in South Asia that is population-based. All population-based data of PCR-confirmed pertussis to-date are from clinical trials conducted primarily in high-income countries with the exception of Senegal. Lastly, all population-based PCR-confirmed pertussis studies published to-date (excluding the Senegal clinical trial) monitored infants after receipt of the first or most commonly the third pertussis vaccination. These studies therefore missed the period in early infancy when infants are at highest risk for morbidity and mortality[7-11]. Our study followed infants from birth through 6 months providing a more complete dataset of pertussis incidence in infancy.

We hypothesized we would find a higher incidence of infant pertussis than found in other settings where population-based active surveillance was used as the burden of childhood pertussis disease is estimated to be highest in South Asia[1]. Further, Nepal provides no pertussis booster doses in adolescents or adults as are routinely given in other settings. Moreover, we expected high delay in receipt of the primary pertussis vaccination series.

Contrary to expectations, our prospective, population-based cohort of infants in Sarlahi, Nepal found a relatively low incidence of pertussis. The finding of low incidence was unexpected based on our original assumptions. However, since our research began, the published literature on pertussis epidemiology and vaccine research has increased dramatically. Reasons for this acceleration of pertussis research include increasing pertussis incidence combined with a move towards maternal pertussis immunization[12]. These recent data supports our findings and provide a potential rationale for the low pertussis incidence observed.

While pertussis incidence has gradually increased in the last two decades, the most recent four years have seen a dramatic jump in cases[13]. Infants and adolescent have experienced the most pronounced pertussis resurgence compared to other age groups. Outbreaks have been reported in multiple high-income countries[14-21]. Several factors have contributed to this increase including the switch from a whole-cell pertussis (wP) vaccine in high resource settings to an acellular pertussis (aP) vaccine[3,22-27]. Data from animal models and humans support the hypothesis of lower efficacy and shorter duration of protection for those partially or exclusively immunized with aP vaccines. The outbreaks have primarily occurred in aP vaccine settings. Our data are consistent as we found low incidence in Nepal's wP vaccine setting. Moreover, low infant pertussis burden was observed despite substantial delays in receipt of the primary pertussis vaccine series and overall low levels of maternal PT antibody levels. Over half of infants who contracted pertussis had received no pertussis vaccinations even though they all were eligible for at least one vaccination by the time of disease

onset. If vaccination delays were minimized and maternal pertussis antibody levels were boosted, we would expect an even lower pertussis incidence in infants.

The symptoms of infants with pertussis were generally milder than that expected by traditional pertussis case definitions. Detection of less severe cases was expected as the study used testing criteria that were more sensitive than previously used. This supports our understanding of pertussis as an underreported disease. However, the public health significance of this under reporting is unclear given the unreported cases are likely to be mild and cause limited morbidity compared to reported cases. These mild cases may play a role in disease transmission to susceptible persons such as vulnerable infants.

Over half of the mothers had PT antibody levels below the lower level of quantification. While PT antibodies were passively transferred to infants, infants often had a lower level of PT antibodies than their mothers. The low prevalence of PT antibodies would support a strategy to increase maternal pertussis antibodies to protect mothers and their infants before they can be vaccinated themselves. An original goal of our study was to support a maternal pertussis vaccination strategy since there were no data or recommendation to vaccinate women during pregnancy. Since our study began there have been at least two randomized trials of aP vaccine in pregnancy of which the results are starting to become available[12,28,29]. In addition, in the U.S. and England, pertussis vaccination was recently recommended in pregnancy[30,31]. The support for these recommendations came not from the ongoing clinical trials but from the high risk infants faced during the pertussis resurgence in these countries. The Advisory Committee on

Immunization Practices (ACIP) advised that the risk of infant pertussis disease outweighed the theoretical risk of vaccine adverse events and should be recommended in all pregnancies[32]. While data are being generated for aP vaccines in pregnancy in high-income countries, data are still lacking to support this strategy in other settings, such as Nepal, that have limited resources and need to invest in strategies for which evidence of cost effectiveness is compelling.

Limitations

While our study had several limitations outlined in the individual studies, some key limitations should be highlighted. First, our infant follow-up ended at 6 months. For comparability of our vaccination timing data to World Health Organization (WHO) reported coverage it would have been ideal to follow infants until at least 12 months of age. While we saw vaccination delay in the first 6 months, extending the surveillance would allow greater understanding of when the vaccines were actually received, given that high coverage is reported nationally at this age.

Second, one of our original aims was to conduct a case-control study identifying pertussis cases and controls and estimating the difference in their and their mother's PT antibody levels at birth. When this idea was originally proposed no published study had examined the association between antibody level at birth and protection from clinical disease. Unfortunately, we were not able to collect blood specimens at birth from any of the infants who contracted pertussis and thus unable to complete this aim. In the interim however, there have been data published demonstrating clinical protection of disease associated with higher in-

fant pertussis antibodies[33]. More data are needed on the efficacy of maternal vaccination in reducing infant pertussis.

A third limitation of our study was that mid-nasal swabs were collected instead of the deeper nasopharyngeal swabs. Our method was non-standard (and based on the needs of the influenza study for the least invasive sampling method) but is supported by preliminary evidence in one study under review[34]. Guidelines for specimen collection were developed for testing by culture, which is significantly less sensitive than PCR. Combined, the theoretical plausibility with the initial data support that we were able to capture all pertussis cases for which we took nasal swabs. However, until further data are collected we cannot be certain that we did not miss cases due to our specimen collection method.

Another important limitation was that our surveillance period for pertussis was limited to a two-year period. Pertussis is a cyclical disease, even in the vaccine era[35]. There is a potential that our surveillance captured pertussis burden during years of lower incidence. A longer surveillance period spanning several years would have been optimal to ensure we captured the peak and nadir of pertussis circulation.

Future Research

While pertussis research has expanded in recent years there is still a need for additional data given pertussis' changing epidemiology and new prevention strategies. Our initial findings lead to several research questions for further study. We have secured additional funding from the Bill and Melinda Gates Foundation

to address some of our limitations and expand our pertussis research questions where indicated.

Future research should include comparing the sensitivity and specificity of PCR using mid-nasal specimens versus nasopharyngeal specimens. With advancements leading to PCR as the assay of choice to diagnosis pertussis there also should be commensurate work into the appropriate sampling region for the more sensitive PCR assay. Currently, health care workers must use the invasive and uncomfortable nasopharyngeal swab or aspirate, which may limit the ease and acceptance of testing in a community-based setting. If comparability studies confirm the initial finding of the acceptability of mid-nasal swabs this could expand the populations for which persons with suspected pertussis are tested thus reducing under diagnosis and under reporting.

Data on the safety, immunogenicity, and efficacy of aP vaccines in pregnancy are needed in a variety of settings. While we expect these data to be available in the near future for Canada and the U.S. there are no known planned studies in South Asia or other low-income settings. If the results from the current trials are in support of pertussis vaccine in pregnancy, efforts should be made to accelerate testing to expand access to this vaccine to low and middle-income countries. The current trials are being conducted in settings where aP vaccines are used exclusively. Further research will be needed on whether an aP vaccine in pregnancy can be coupled with a wP childhood vaccination series. The WHO already recommends tetanus toxoid vaccine in pregnancy so adding an additional target (acellular pertussis) to this vaccine would not add an additional immun-

ization in pregnancy, or at least would not add another antenatal care visit for vaccination.

With emerging evidence showing that the aP vaccine has low efficacy and limited duration of immunity, researchers in vaccine development should work towards a vaccine that has a similar safety profile to the aP paired with the effectiveness of the wP vaccine. A proliferation of studies in the past two years demonstrates the acceleration of pertussis vaccine development[36-46].

Vaccine delay for pertussis vaccination was common in our study population. Groups at higher risk for non-vaccination in Nepal were children born SGA, mothers who delay initiation of breastfeeding, and mothers of Madeshi ethnicity. Further research is needed on which policies or interventions are effective in improving timely vaccination uptake.

Our study contributes to the growing knowledge that the WHO metric of vaccination coverage does not adequately measure delays in time to pertussis vaccination nor do reported pertussis cases capture the true burden of disease. Research into methods to improve surveillance of pertussis disease and vaccine coverage should be pursued. A limitation of our study was that we only captured two years of surveillance. Additional funding will now allow us to test all of the nasal swabs collected during the larger trial, expanding our observation period to approximately 3 years. Data from a third year will decrease the possibility that our surveillance period was during a time of low pertussis incidence.

Our original funding covered testing of antibodies to only one pertussis antigen, pertussis toxin (PT). Funding from the Bill and Melinda Gates Foundation will allow us to conduct ELISA testing on three additional pertussis antigens – filamentous hemagglutinin (FHA), pertactin (Prn), and fibriae (FIM). Since no one antigen can be correlated to pertussis immunity, having antibody levels to the four antigens most commonly used in acellular pertussis vaccines will provide a more comprehensive set of data on maternal and infant pertussis-related antibodies.

In addition to passive transfer of antibodies through the placenta during gestation infants are also able to receive maternal antibodies during breastfeeding in infancy. There are limited data on the level of PT breastmilk IgA and its role in infant protection[47-51]. Supplemental funding will allow us to test previously collected breastmilk samples from the same women who we have already tested at the delivery time-point blood for pertussis antibodies in breastmilk. While we will not be able to correlate breastmilk antibody levels with infant pertussis disease we will be able to look at the association of breastmilk antibody with maternal serum antibody levels.

Policy Implications

Several recommendations to policy makers in Nepal follow from this research. First, increased emphasis is needed on vaccination timeliness in the first year of life as a complement to high vaccination coverage at 12-23 months. Our data show there is substantial delay for pertussis containing vaccines that likely extends to other childhood immunizations. While children are unimmunized they

are at unnecessary increased risk for contracting vaccine-preventable diseases. Our research indicates specific groups could be targeted for vaccine outreach to improve vaccine uptake and understand in greater detail Nepal-specific barriers to vaccination. Concurrently, while there are logistical challenges to monitoring for pertussis such as the availability of trained clinicians with the ability to test for pertussis and equipped laboratories for performing the assays, investments should be made in disease surveillance infrastructure.

We found a low burden of pertussis in infants less than 6 months of age in Nepal. One conclusion from this could be that the current vaccine is effective in preventing pertussis through herd immunity, despite delays in receiving the vaccines. In the recent past some countries were considering a switch from whole cell pertussis vaccines to acellular pertussis vaccines. Nepal should follow recently released WHO recommendations to continue using the whole cell pertussis vaccine in light of data showing its superior efficacy compared to acellular pertussis vaccines [52]. Another consideration for switching to the acellular vaccine would be an increased cost compared to the whole cell vaccine.

An original aim of this research was to provide foundational data for future studies on maternal pertussis vaccination in low-income settings. Given our findings of low pertussis incidence and low levels of maternal pertussis antibody the decision to introduce pertussis vaccination in pregnancy is not clear. First, the results from studies in the U.S. and Canada have not been fully published so we lack a complete understanding of their safety and immunogenicity in pregnancy. If the final results from these two trials are supportive of this strategy it would be

important to gather local data in Nepal or a similar country to understand if the vaccine would continue to have positive outcomes in a different population. If the vaccine was found effective in the Nepalese population, policy-makers would have to consider whether the disease burden warrants introduction of a new vaccine in pregnancy, especially given it would be a permanent increased cost to the government or external funding agency[53]. Logistically, Nepal has an existing framework for vaccination during pregnancy with the tetanus toxoid vaccination program. Combination vaccines exist, which include both of these antigens, so an additional vaccination would not be necessary. The pertussis study was nested within a trial of influenza vaccination during pregnancy. The government of Nepal will likely use the pending results from this trial to inform a decision of whether to include influenza vaccination in its maternal vaccination program. The Nepal government's decision-making with influenza vaccine will likely influence its future desire to support and recommend new vaccines.

Conclusion

Substantial delays in pertussis vaccination were found in the first 6 months of life that are not captured by WHO vaccination coverage estimates. Over half of mothers and their infants had PT antibody levels too low to be quantified. Some infants had antibody levels lower than their mothers indicating not all gestational transfer was active. Despite vaccine delay and low antibody seroprevalence we found low risk for pertussis among infants in rural Nepal. The duration and severity of pertussis disease was generally milder than expected based on traditional clinical findings. Even with delays the whole cell pertussis vaccine appears

to be effective in protecting infants from pertussis. The epidemiology of pertussis should be monitored for increasing incidence with maternal pertussis vaccination as a promising strategy if the disease burden increases.

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erature. *Early human development*. 2011;87(2):67–72.

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young infants: a review of key evidence informing targeting of the cocoon strategy. *Vaccine*. 2013;31(4):618–625. doi:10.1016/j.vaccine.2012.11.052.

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World Health Organization. *Laboratory Manual for the Diagnosis of Whooping Cough Caused by Bordetella Pertussis/Bordetella Parapertussis*. World Health Organization; 2014.

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Zackrisson G, Taranger J, Trollfors B. History of whooping cough in nonvaccinated Swedish children, related to serum antibodies to pertussis toxin and filamentous hemagglutinin. *The Journal of pediatrics*. 1990;116(2):190–194.

Zhang L, Prietsch SO, Axelsson I, Halperin SA. Acellular vaccines for preventing whooping cough in children. *Evidence-Based Child Health: A Cochrane Review Journal*. 2011;(1):CD001478.

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Zouari A, Smaoui H, Brun D, et al. Prevalence of Bordetella pertussis and Bordetella parapertussis infections in Tunisian hospitalized infants: results of a 4-year prospective study. *Diagnostic microbiology and infectious disease*. 2012;72(4):303–317. doi:10.1016/j.diagmicrobio.2012.01.002.

MICHELLE M. HUGHES

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Chicago, IL 60647

EDUCATION

PhD Johns Hopkins Bloomberg School of Public Health 2010 - present
International Health: Global Disease Epidemiology and Control
Dissertation: "Characterization of Pertussis Risk and Antibody Transfer in Infants in Sarlahi District, Nepal: A Community-Based Prospective Cohort Study"
Committee: Joanne Katz, James Tielsch, Mark Steinhoff, and Saad Omer

MHS Johns Hopkins Bloomberg School of Public Health May 2009
International Health: Global Disease Epidemiology and Control
Advisor: Saad Omer and Joanne Katz
Graduated with 4.0 GPA

BA Washington University, Biology and Anthropology May 2006
Graduated Cum Laude

School for International Training Study Abroad 2004
Kenya Development, Health, and Society
China: Public Health, Traditional Chinese Medicine, and Mandarin

HONORS AND AWARDS

Lancet/Consortium of Universities for Global Health Student Poster Award 2014
Award for poster presentation on pertussis dissertation research. [$500]

David and Elinor Bodian Scholarship Award 2014
Scholarship given to doctoral student to honor the work of Dr. Bodian, who laid the scientific groundwork for the development of the polio vaccine. [$9,500]

Procter & Gamble Fellowship 2013
Fellowship awarded to doctoral student committed to advancing the health and well-being of women and children. [$20,000]

Global Health Field Research Award 2012
Scholarship to support dissertation field research in Nepal. [$2,500]

R. Bradley Sack Family Scholarship Award 2012
Scholarship for outstanding doctoral student studying infectious disease in the developing world. [$4,350]

Clements-Mann Award 2010
Award given to outstanding graduate student working in vaccine sciences. [$5,000]

Clinical Trials Training Program, National Eye Institute 2010
Pre-doctoral National Research Service Award (NRSA) trainee through the Department of Epidemiology. [Full tuition, stipend, and health insurance]

Assistant Secretary for Health's Award for Outstanding Team Performance 2010
For outstanding service with the Immunization Safety Task Force H1N1 Working Group and for achieving unprecedented level of vaccine safety monitoring.

Delta Omega Honorary Society for Public Health 2009
Inducted in the Alpha Chapter of the Delta Omega Honorary Society for Public Health.

Vaccine Science and Policy Certificate 2009
Completed requirements at Johns Hopkins School of Public Health for competency in vaccine science and policy, from clinical research to implementation in the United States and internationally.

John Snow Inc. Award in International Health 2008
Award for outstanding second year student engaged in a field internship. [$1,250]

Global Field Experience Fund Award 2008
Scholarship to support field experience for masters practicum. [$2,000]

Sigma Xi Scientific Research Society 2006

Washington University Anthropology Excellence in Research Award 2006

Captain of Washington University's Women's Club Ultimate Frisbee 2005
Planned practices, coordinated team travel for tournaments, maintained group website, organized home tournament hosting over 25 college teams, and provided overall coaching and leadership for teammates.

Lambda Alpha National Anthropology Honor Society 2004

School for International Training Asia Fund Scholarship 2004
Scholarship for China study abroad program based on academic performance and ability to carry out independent field study. [$3,100]

School for International Training Fund 2004
Scholarship to support Kenya study abroad program. [$1,500]

Washington University Dean's List 2004 - 2005

RESEARCH EXPERIENCE

Dissertation, Johns Hopkins Bloomberg School of Public Health 2011 - present
Advisor: Dr. Joanne Katz
"Pertussis in Infants: Characterization of Risk and Maternal Antibody Transfer in Rural Nepal"

- Developed thesis proposal into E.W. "Al" Thrasher Award ($296,224)
- Coordinated specimen collection, storage, shipping, and testing
- Led data management, analysis and writing of results for presentations and publications

Johns Hopkins Bloomberg School of Public Health, Baltimore, MD 2012 - present
Student Investigator, Maternal Influenza Vaccine Trial, Dr. Joanne Katz

- Led completion of comprehensive responses to external monitoring of trial
- Aided in maintaining compliance with Good Clinical Practice (GCP)
- Developed and maintained Standard Operating Procedures (SOP), Guidelines, a standardized Manual of Operations

New York City Department of Health & Mental Hygiene, New York City, NY 2008
Epidemiology Scholar, Bureau of Communicable Disease, Dr. Trang Ngyuen

- Researched epidemiological disparities in emergency department utilization and influenza vaccination using syndromic surveillance and yearly health survey data using SAS, SUDAAN, SQL, and GIS for analysis
- Presented oral report of findings to Bureau of Communicable Disease

Johns Hopkins Bloomberg School of Public Health, Baltimore, MD 2008
Research Assistant, Dr. Saad Omer

- Performed literature review of over 5,000 articles on pertussis morbidity and mortality for the World Health Organization's Global Burden of Disease

National Cancer Institute, Bethesda, MD 2006 - 2007
Cancer Research Training Award Fellow, Drs. John Schiller and Douglas Lowy

- Studied the role of L2 minor capsid protein in human papillomavirus (HPV) cell entry and uncoating
- Laboratory techniques: hybridoma library preparation, cloning, ELISA, and HPV pseudovirus production

Washington University, Saint Louis, MO 2005 to 2006
Research Assistant, Biology Department, Dr. Michael Neff

- Studied role of RGL2 and LEP proteins in *Arabidopsis* germination
- Laboratory techniques: DNA sequencing, PCR, mutagenesis screens, and tissue culture

Youth Technology and Education Center, Saint Louis, MO 2005
Survey Intern

- Developed and administered educational needs-assessment survey
- Analyzed data and documented findings for use in future grant writing

Washington University School of Medicine, Saint Louis, MO 2002 to 2004
Research Assistant, Molecular Diagnostics Laboratory, Dr. Barbara Zehnbauer

- Studied genetic markers as predictors of predisposition to severe sepsis
- Laboratory techniques: PCR, sequencing, and TDI (method to detect single nucleotide polymorphisms)

TEACHING EXPERIENCE

Johns Hopkins School of Public Health, Baltimore, MD 2009
Teaching Assistant, International Health Department, Dr. James Tielsch

- Provided detailed feedback and assessment of over 50 students' course papers for the course "Introduction to International Health"

University Teaching 101, Coursera 2014
Student, Johns Hopkins University

- Completed six-week online course on the foundational knowledge of the science of teaching and learning as well as skills and strategies for teaching at the University level.

WORK EXPERIENCE

Sinai Urban Health Institute, Chicago, IL 2014 - present
Senior Epidemiologist

World Health Organization, Geneva, Switzerland 2013- 2014
Consultant Rapporteur, Strategic Advisory Group of Experts (SAGE) on Immunization

- Provided meeting minute support to the Secretariat for the Pertussis Working Group and other SAGE-associated meetings

U.S. Department of Health & Human Services, Washington, DC 2009 - 2010
Policy Fellow, National Vaccine Program Office

- Coordinated government's response to the H1N1 influenza pandemic through work with the Federal Immunization Safety Task Force and the H1N1 Vaccine Safety Risk Assessment Working Group of the National Vaccine Advisory Committee
- Led revision of the vaccine safety goal for the 2010 National Vaccine Plan

Catholic Relief Services' AIDSRelief Program, Lusaka, Zambia 2008
Monitoring and Evaluation Intern

- Developed, administered, and analyzed over 50 questionnaires to investigate high HIV-related patient mortality at clinics
- Documented task shifting of nurses' duties to community health workers at HIV clinics in a report for AIDSRelief partners and donors
- Trained staff at HIV treatment sites to conduct a quality improvement study including administration of an anti-retroviral adherence survey and collection of blood samples for viral load testing

PUBLICATIONS

Journal Publications

Hughes, M.M., Katz, J., Mullany, L.C., et al., "Seasonality of Birth Outcomes in Rural Sarlahi District, Nepal: A Population-Based Prospective Cohort," BMC Pregnancy and Childbirth, vol.14, no.310, 2014. doi:10.1186/1471-2393-14-310.

[Previous published under Michelle J. Mergler]

Mergler, M.J., Omer, S.B., and Pan, W.K., et al. "Association of Vaccine-Related Attitudes and Beliefs between Parents and Health Care Providers," Vaccine, vol. 31, no. 41, 2013, pp. 4591-4595.

Mergler, M.J., Omer, S.B., and Pan, W.K., et al. "Are Recent Medical Graduates More Skeptical of Vaccines?," Vaccines vol. 1, no. 2, 2013, pp. 154-166.

Salmon, D.A., Akhtar, A., **Mergler, M.J.,** et al. "Immunization Monitoring Systems for the 2009 H1N1 Monovalent Influenza Vaccination Program," Pediatrics, vol. 127, pp. S78 – S86.

Mergler, M.J., "Factors Influencing Family Planning Usage Among Women in Takaungu of Kilifi District in Kenya," *Apex*, Washington University College of Arts and Sciences, vol. 1, Fall 2004, pp. 59-82.

PRESENTATIONS

Poster Presentation, "Pertussis in Infants: Characterization and Maternal Antibody Transfer in Rural Nepal," Consortium of Universities for Global Health, Washington, DC. May 10, 2014.

Platform Presentation, "Pertussis in Infants? Characterization of Risk and Maternal Antibody Transfer in Rural South Asia," Pediatric Academic Societies, Vancouver, Canada. May 4, 2014.

Poster Presentation, "Pertussis in Infants: Characterization of Risk and Maternal Antibody Transfer in Rural Nepal", Johns Hopkins Delta Omega, Baltimore, MD. February 20, 2014.

Poster Presentation, "Are Younger Doctors More Skeptical of Vaccines? Evaluation of a Provider Cohort Effect Regarding Immunization Beliefs", Infectious Disease Society of America, Boston, MA. October 21, 2011.

Oral Presentation, "Association of Vaccine-Related Knowledge, Attitudes, and Beliefs Between Parents and Health Care Providers", National Immunization Conference, Atlanta, GA. April 21, 2010.

Poster Presentation, "Generation of Monoclonal Antibodies that Recognize Different Human Papillomavirus (HPV) Capsid Conformations", National Institutes of Health Post-Baccalaureate Research Festival, Bethesda, MD. May 9, 2007.

PROFESSIONAL TRAINING

Certified in Public Health, National Board of Public Health Examiners, 2012 - present

PROFESSIONAL SERVICE

Peer-Reviewed Articles for:

- Pediatrics (2012-2014)
- Vaccine (2014)

COMMUNITY SERVICE

Green Student Group

Founder, Johns Hopkins Bloomberg School of Public Health, 2011

Formed new student group to facilitate action to increase school-wide environmental sustainability. Activities included a letter to the Dean and a petition to ban bus idling, support green catering, and support single-stream recycling.

Science Outreach

Teaching teams classroom leader, Washington University, 2003-2006

Led Washington University students to develop and lead exciting experiments in physics, chemistry, anthropology, and biology weekly to foster a love of science in underprivileged students.

LANGUAGES

English: Native Language

French: Novice Listener, Novice Speaker, Intermediate Reading and Writing

COMPUTER SKILLS

Statistical/Programming: Stata, R, SAS, GIS, SQL, SUDAAN

Applications: Microsoft Suite (Word, Excel, PowerPoint, Access), EndNote, Reference Manager, RefWorks, Papers

OTHER

U.S. Citizen